

## SPERMATOGENESIS IN *PARATETTIX*.<sup>1</sup>

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Wilson has said that "heredity is a consequence of the genetic continuity of cells by division, and the germ cells form the vehicle of transmission from one generation to another."

If this be true we should look to the structure of the germ-cells for an explanation of the phenomena that have been and are being found out in heredity. Cytologists have discovered much concerning the structure of the germ-cells and the behavior of the chromosomes during the processes of maturation and division. The combined knowledge of sex and sex ratio, and the cytological constitution of germ-cells has shown in many forms, at least, a correlation between the inheritance of sex and the dimorphism of spermatozoa or eggs, or both. However, the vast amount of cytological work has been done with forms the behavior of whose characteristics in heredity is unknown. On the other hand, much of the work in heredity has been done with forms of which little or nothing is known of the structure of the germ-cells. It is the writer's good fortune to have access to material of which some of the ancestry is known for eighteen generations, covering a period of five years.

For a number of years Dr. R. K. Nabours<sup>2</sup> has been conducting experiments with regard to inheritance in *Paratettix*, a genus of the short-horned grasshoppers. The characteristics used in his investigations are the color patterns of the pronotum and femora of the jumping legs, and the lengths of the pronotum and wings. The data show that the inheritance of the color patterns is Mendelian in its behavior. In the F<sub>1</sub> hybrid no part of the color pattern of one parent species is ever replaced by the color pattern of the other parent, but the color patterns of both parents are present. Reciprocal crosses give identical results. The

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<sup>2</sup> The writer wishes to thank Dr. R. K. Nabours for the grasshoppers which have furnished the material for this paper.

lengths of the wings and pronotum are not inherited but are closely correlated with the length of time required for the animal to reach maturity. These grasshoppers have furnished the material for the present paper.

The work on the cytological constitution of the germ-cells of *Paratettix* has been undertaken for the purpose of discovering whether or not the microscope will reveal any differences in the germ-cells of very closely related forms which may be correlated with the differences in the color pattern. The spermatogenesis of only one form (*Paratettix leuconotus-leucothorax*) is given here. *Paratettix leuconotus-leucothorax* is a hybrid, obtained by crossing *P. leuconotus* with *P. leucothorax* (or by the interbreeding of two hybrids, one being a hybrid of *leuconotus* with some other form and the other a hybrid of *leucothorax* with some other form). No attempt has been made to show any relation between the structure of the germ-cells and the somatic structures. This will be discussed in a later paper.

The chromosomal complex of the spermatogonia of *Paratettix leuconotus-leucothorax* consists of thirteen rod-shaped bodies which may be divided into two groups—one group consisting of four larger chromosomes and the other of nine smaller ones. Neither the larger nor the smaller chromosomes form equal sized pairs as Sutton has found in *Brachystola magna* and which is so frequently described for the Hemiptera and is apparently characteristic of all Diptera. All of the large chromosomes and one of the small ones are bent rods or slightly U-shaped, but the other eight are almost straight. No one of these chromosomes has been surely identified as the accessory. However, in the early prophases there is always present a mass of chromatin which has a more compact consistency and stains more intensely than the remainder of the chromatin (A, Fig. 1). This mass has not been identified with any chromosome nor is it associated with a vesicle as described by Carothers for *Arphia simplex*. There appears to be no difference in the staining capacity nor in the compactness of the chromosomes in the late prophases (Fig. 2).

The spermatogonial spindle is long and slender, and has fine but distinct fibers which converge at the poles. The centrosome



which is very distinctly visible in the metaphase stage is small and spherical. It stains almost as intensely as the chromosomes. The astral rays are short and indistinct. The chromosomes are at right angles to the spindle fibers in the metaphase stage (Fig. 7). A metaphase plate always shows one chromosome nearer the center of the spindle than any other chromosome. Sometimes it is completely surrounded by the others (Figs. 4 and 6) and sometimes merely one end is at the center of the spindle (Figs. 3 and 5). This chromosome is always one of the larger of the group of smaller ones but it is never the bent one. Few anaphase and no telophase stages have been observed. Fig. 8 shows an anaphase with rather indistinct spindle fibers, which is characteristic of all the anaphase stages observed. The centrosome, which shows distinctly in the metaphase stage (Fig. 7), is now invisible. The chromosomes are no longer at right angles to the spindle fibers but are nearly parallel with them.

Fig. 9 illustrates the condition of the cell at the beginning of the growth period. The nucleus is large and comparatively clear. Some of the chromatin is in a finely reticular condition and stains faintly with iron-haematoxylin. However, a mass of the chromatin retains the compact consistency and the density of stain of the chromosomes (A, Fig. 9). It has a rounded form like a nucleolus. The boundary between the nucleus and the cytoplasm is quite evident. The nucleus continues to increase in size and the reticular chromatin, which now has a greater staining capacity, forms a thread or threads having a woolly appearance. There is no polarization of the loops of the spireme but they occupy almost all of the space of the nucleus and form an irregular tangled mass. The nucleolus remains at one side of the nucleus and does not have the woolly appearance that the spireme has (Fig. 10).

In the synezeis or contraction stage, the spireme seems to shrink away from the nuclear wall, leaving a clear space between the cytoplasm and the chromatin material. There is little difference between the character of the chromatin and its staining capacity in this stage and the preceding one. The compact mass of chromatin never loses its identity and always remains at one side of the nucleus (Fig. 11). The boundary of

the nucleus soon becomes irregular, and the chromosomes of the primary spermatocyte is formed by a breaking up of the spireme thread into segments. The compact intensely staining mass which has been traced through the growth period is shown in Fig. 12 as a chromosome which differs in shape from the other chromosomes. It is ovoid and without a constriction in the middle, while all the other chromosomes are dumb-bell shaped. Not all of the chromosomes are formed at the same time. The chromatin retains its loose woolly appearance until after it has broken up into parts, then it gradually becomes more compact, takes the stain more readily and each part assumes the characteristic dumb-bell shape. While this is taking place the boundary between the nucleus and the cytoplasm becomes more irregular and by the time the chromosomes are completely formed the cytoplasm has formed a vesicle around each of them (Figs. 13 and 14).

The chromosomal complex of the primary spermatocyte consists of six dumb-bell shaped chromosomes and one ovoid chromosome. Of the six dumb-bell chromosomes two are decidedly larger than the others and one of these is much larger than the other one, as is shown in Figs. 13 to 16 inclusive. The ovoid or accessory chromosome is never among the other chromosomes but always lies near the periphery of the nucleus as it did in the growth period. When the chromosomes have become arranged on the spindle the dumb-bell chromosomes are well toward the center of the spindle, while the accessory is always near the periphery. It does not remain long in the metaphase plate but soon passes toward one pole undivided much in advance of the other chromosomes. For this reason many sections of metaphase plates show only six chromosomes and those which show seven are often cut obliquely. Not all of the chromosomes in the primary spermatocyte divide synchronously. Fig. 20 shows five of the dumb-bell chromosomes divided while the largest one shows little constriction. This division is transverse as is shown in Figs. 16 and 20. There are no loops, rings, or U's which would give the least indication of a longitudinal division. In the metaphase or early anaphases the centrosome is a small spherical body and takes the stain readily. The

spindle fibers are fine but distinct. The astral rays are similar to those of the spermatogonial divisions. In the late anaphases the centrosome is no longer visible and the spindle fibers are indistinct. There seems to be no resting stage between the first maturation division and the second maturation division.

The chromosomes of the second spermatocyte are ovoid. Metaphase plates show six and seven chromosomes (Figs. 23 and 24). The accessory cannot always be distinguished from the other chromosomes. It is either the second or the third largest. It divides in this division and passes to the poles in advance of the others (Fig. 25). In the late anaphases all the chromosomes have coalesced, although the number may yet be distinguished (Fig. 26). By the time the chromosomes have reached the poles they form a diffuse mass of chromatin at each pole, and the cell has begun to constrict in the middle.

The centrosome which is similar to the centrosome of the primary spermatocyte has disappeared. The spindle fibers have become indistinct. As the constriction of the cell is completed the chromatin has migrated to the center of each daughter cell.

In the changing of the spermatid into the spermatozoön two things are conspicuous from the beginning, the changes in the character of the chromatin and the elongation of the cell body. From the diffuse irregular mass there is formed an ovate body with the chromatin in a coarsely reticular condition largely around the periphery of it. The cell becomes elongate and larger at one end than at the other. The cytoplasm has changed from the tangled network to fibrillar strands of granules extending longitudinally across the cell. The cell wall is quite distinct. This condition is illustrated in Fig. 29. The spermatid continues to elongate. The nucleus becomes spherical and remains at one end of the spermatid. The more granular cytoplasm lies toward the periphery of the tail-like elongation. There is a portion of the cytoplasm extending from the nucleus through the center of the tail which is more finely granular than the remainder and takes the stain less readily. Part of the chromatin has become massed together, forming a sphere situated to the side of the nucleus near the end of the lightly staining area of the tail. The greater part of the remaining chromatin is dis-

tributed around the periphery of the nucleus (Fig. 30). As the tail becomes longer it becomes thinner and a filament extends from the nodule of the head through the entire length of the tail. Very little cytoplasm now surrounds the head (Fig. 31). Finally the head becomes arrow shaped and stains very intensely. The tail is long and filamentous and stains a little less intensely than the head. The head and a portion of the tail are illustrated by Fig. 32. The tail is more than four times as long as is shown in the figure.

McClung ('14) says: "It seems very evident that in the spermatogonia of the Acrididæ we are dealing with a chromosome complex of a very definite and precise organization which, in the great majority, presents itself without essential variation in number, size and form, fiber attachment, arrangement in the metaphase and behavior during division of its elements. *Stenobothrus* and *Pamphagus* seem to be definite exceptions in some of these respects. . . ." And again he says:

"With the exception of the *Stenobothrus*-like species, and *Pamphagus*, the students of the Acrididæ have reported a reduction of the 23 spermatogonial chromosomes to 12."

All of the genera of the family Acrididæ<sup>1</sup> discussed in McClung's paper belong to the three subfamilies, Tryxalinæ, Ædipodinæ, and Acridinæ, and none belong to the subfamily Tettiginæ. It would seem that with the general agreement of the great numbers of genera of this family which he and his students have studied as well as those of other independent workers that he would be justified in making the general statements concerning the family. However, *Paratettix leuconotus-leucothorax* of the subfamily Tettiginæ, show some exceptions. The spermatogonial number of chromosomes are thirteen instead of twenty-three. The number of chromosomes in the primary spermatocyte is seven. This the writer has found to be true also for both the parent forms of the hybrid as well as for others belonging to the genus *Paratettix*.

<sup>1</sup> The writer is aware of the fact that there has been much shifting about of names of the short-horned grasshoppers and that some taxonomists consider the grouse locusts of family value. If this should be the position which McClung takes, then he would not consider *Paratettix* as belonging to the family Acrididæ and it would follow that the observations recorded in this paper would not be exceptions to his statements concerning Acrididæ.

Robertson ('15) says: "In the Tettigidae (Tettiginæ) a subfamily of the short-horned grasshopper family Acrididæ, I have found for all the specimens of at least four different genera which I have examined the number of chromosomes to be uniformly 14 in the female and 13 in the male."

From the above data one would scarcely be justified in saying that the characteristic number of spermatogonial chromosomes of the subfamily Tettiginæ is thirteen but the writer feels justified in saying that this is an essential variation in the number of chromosomes given in the above quotation from McClung as the number characteristic for the family Acrididæ. The writer has found no indication of multiple chromosomes.

In the prophase tetrad six of the chromosomes are always dumb-bell-shaped and one ovoid. There are none of the irregular shaped chromosomes as described by McClung and no indications of the annular chromosomes which he says "that practically without exception every investigator of recent years who has made a careful study of the maturation stages in the Orthoptera has seen and figured." If the dumb-bell-shaped chromosomes are similar to his I-shaped chromosomes they differ in that they do not have an enlargement in the middle, but rather they have the appearance of a constriction. This constriction is not due to the initiation of the division, for it is present before the chromosomes are arranged on the spindle; in fact, they have their characteristic shape before the spindle is visible.

The presence of a mass of chromatin in the resting stage of the spermatogonial divisions which is of a different form and different staining capacity and also the presence of a similar mass in the growth period, which can be identified as the accessory chromosome of the spermatocyte, gives added evidence for the continuity of chromosomes as definite entities.

The spermatozoön of *Paratettix leuconotus-leucothorax* is very different from the spermatozoön of *Paratettix cuculatus* as described by Hancock. He describes and figures the head of *P. cuculatus* as being small, thin, and acutely pointed. In fact, from his figure one would think that the head is very little thicker than the tail. He says that the middle piece is formed into a



high, rather short, protoplasmic keel. No keel has been observed as forming a part of the middle piece of *P. leuconotus-leucothorax*. The head is many times thicker than the tail and is decidedly arrow shaped. The middle piece seems to continue from the head to the long filamentous tail without a definite dividing line between them.

## SUMMARY.

1. *Paratettix leuconotus-leucothorax* has thirteen spermatogonial rod-shaped chromosomes, four larger and nine smaller ones.

2. Neither the larger nor the smaller chromosomes form equal sized pairs.

3. The four larger chromosomes and one of the smaller ones are bent rods, the others are almost straight.

4. Neither spermatogonial chromosome has been surely identified as the accessory chromosome.

5. In the growth period is a mass of chromatin which is more compact and stains more intensely than the remainder of the chromatin. This is the accessory chromosome of the first spermatocyte.

6. The first spermatocyte chromosomes are formed in vesicles.

7. There are six dumb-bell-shaped bivalent chromosomes and one ovoid univalent chromosome in the primary spermatocyte.

8. The accessory is near the periphery of the nucleus and passes to one pole undivided slightly in advance of the other chromosomes in the first spermatocyte division.

9. The bivalent chromosomes divide crosswise.

10. The accessory chromosome divides in the second division.

11. The spermatozoön has an arrow-shaped head and a long filamentous tail.

## BIBLIOGRAPHY.

Artom, C.

'09 Cromosomi ed eterocromosomi nelli cinesi spermatogenetiche di *Stauronotus maroccanus* Thumb. *Biologica*, Vol. 2.

Buchner, P.

'09 Das accessorische Chromosom in Spermatogenese und Oogenese der Orthopteren, Zugleich ein Beitrag zur Kenntnis der Reduktion. *Archiv für Zellforschung*, Bd. 3, Hft. 3.

Brunelli, Gustavo.

'10 La Spermatogenesi della *Tryxalis*. Divisioni Spermatogoniali. *Memorie della Società Italiana Scienze*, Tome 16, serie 3a.

- '11 La Spermatogenesi della *Tryxalis*. Divisioni maturative. Reale Accademia dei Lincei, Tome 8, serie 5a.
- Carothers, Eleanor.**
- '13 The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes. Journ. Morph., Vol. 24, No. 4.
- Davis, H. S.**
- '08 Spermatogenesis in Acrididæ and Locustodæ. Bull. Museum Comp. Zoöl., Harvard College, Vol. 43, No. 2.
- Gerard, Pol.**
- '09 Recherches sur la Reduction Karyogamique dans la Spermatogenese de *Stenobothrus biguttulus* (L.). Bull. Société Roy. Sciences med. nat. de Bruxelles, No. 1.
- '09 Recherches sur la Spermatogenese chez *Stenobothrus biguttulus* (Linn.). Archives de Biologie, Tome 24.
- Granata, Leopoldo.**
- '10 La cinesi Spermatogenetische di *Pamphagus marmoratus* (Brum). Archiv für Zellforschung, Bd. 5, Hft. 2.
- Hancock, J. L.**
- '02 The Tettigidae of North America. The Lakeside Press. R. R. Donneley & Sons Company, Chicago.
- Hartman, Frank.**
- '13 Variations in the Size of Chromosomes. BIOL. BULL., Vol. 24, No. 4.
- McClung, C. E.**
- '00 The Spermatocyte Divisions of the Acrididæ. Kansas U. Quarterly, Vol. 9, No. 1.
- '02 The Accessory Chromosome—Sex Determinant? BIOL. BULL., Vol. 3, Nos. 1-2.
- '05 The Chromosome Complex of Orthopteran Spermatocytes. BIOL. BULL., Vol. 9, No. 5.
- '08 Cytology and Taxonomy. Kansas U. Science Bull., Vol. 4, No. 7.
- '14 A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. Journ. Morph., Vol. 25, No. 4.
- Meek, C. F. Y.**
- '11 The Spermatogenesis of *Stenobothrus viridulus*, with Special Reference to the Heterotropic Chromosome as a Sex Determinant in Grasshoppers. Linnean Society's Journ. Zoöl., Vol. 32, No. 208.
- '12a A Metrical Analysis of Chromosome Complexes, showing correlation of Evolutionary Development and Chromatin Thread-width Throughout the Animal Kingdom. Philosoph. Transactions Roy. Soc. of London, Series B., Vol. 203.
- '12b The Correlation of Somatic Characters and Chromatin Rod-lengths, being a further study of Chromosome Division. Linnean Society's Journ. Zoöl., Vol. 32.
- Metz, Charles W.**
- '14 Chromosome Studies in the Diptera. I. A Preliminary Survey of Five Different Types of Chromosome Groups in the Genus *Drosophila*. Journ. Exper. Zoöl., Vol. 17, No. 1.
- Montgomery, T. H., Jr.**
- '05 The Spermatogenesis of *Syrbula* and *Lycosa*, with general Considerations

upon Chromosome Reduction and the Heterochromosomes. Proc. Academy Nat. Sci. Philadelphia, February.

**Nabours, R. K.**

- '14 Studies of Inheritance and Evolution in Orthoptera, I. Journ. of Genetics, Vol. 3, No. 3.

**Nowlin, Nadine.**

- '08 The Chromosome Complex of *Melanoplus bivittatus* Say. Kansas U. Sci. Bull., Vol. 4, No. 12.

**Pinney, Edith.**

- '08 Organization of the Chromosomes in *Phrynotettix magnus*. Kansas U. Sci. Bull., Vol. 4, No. 14.

**Robertson, W. R. B.**

- '08 The Chromosome Complex of *Syrbula admirabilis*. Kansas U. Sci. Bull., Vol. 4, No. 13.

- '15 Chromosome Studies. III. Inequalities and Deficiencies in Homologous Chromosomes; their Bearing upon Synapsis and the Loss of Unit Characters. Journ. Morph., Vol. 26, No. 1.

**Wilson, E. B.**

- '13 Heredity and Microscopical Research. Science, N.S., Vol. 37, No. 961.

## EXPLANATION OF PLATES.

All figures were made at table level by means of a Zeiss compensating ocular No. 6 and a 1.5 mm. objective with the aid of a camera lucida. The drawings were enlarged two diameters and then reduced one third.

## PLATE I.

FIG. 1. Early prophase of spermatogonial division. *A*, a mass of chromatin which does not become reticular but remains more or less compact.

FIG. 2. Formation of spermatogonial chromosomes.

FIGS. 3 TO 6. Metaphase plates of spermatogonial chromosomes.

FIG. 7. Metaphase, lateral view, spermatogonial division showing position of the chromosomes on the spindle.

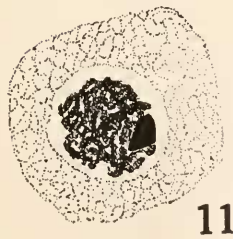
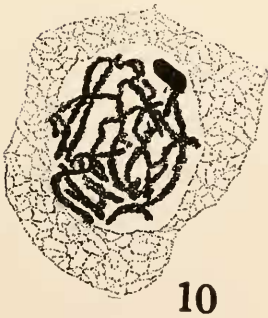
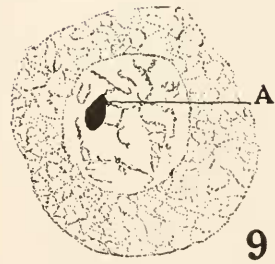
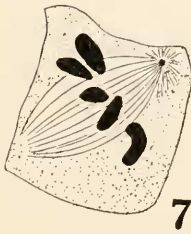
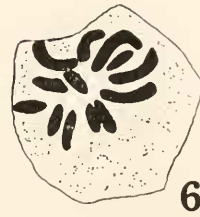
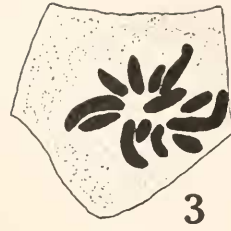
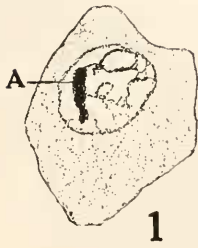
FIG. 8. Anaphase of spermatogonial division.

FIG. 9. Beginning of the growth period. *A*, a mass of chromatin which does not pass into a reticular condition and forms the accessory chromosome.

FIG. 10. Formation of chromatin thread.

FIG. 11. Synizesis.

FIG. 12. Beginning of the formation of the primary spermatocyte chromosomes.









## PLATE II.

FIG. 13. Primary spermatocyte chromosomes showing the beginning of the formation of the vesicles.

FIG. 14. Primary spermatocyte chromosomes in vesicles.

FIGS. 15 AND 16. Metaphase, lateral view, of first spermatocyte division. The accessory chromosome is at the periphery of the spindle.

FIGS. 17 AND 19. Metaphase plates of first spermatocyte division showing seven chromosomes.

FIG. 18. Metaphase plate of first spermatocyte division showing six chromosomes. The accessory chromosome is not in the metaphase plate.

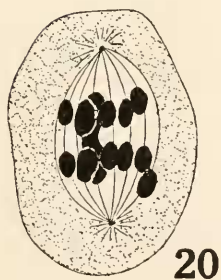
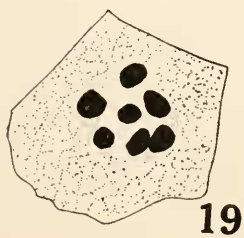
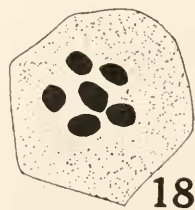
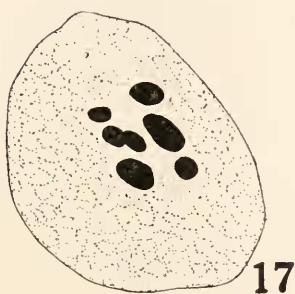
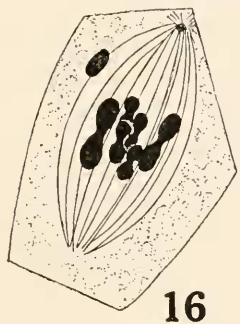
FIG. 20. Beginning anaphase of first spermatocyte division.

FIG. 21. Late anaphase of first spermatocyte division showing seven chromosomes.

FIG. 22. Metaphase, lateral view, of second spermatocyte division showing seven chromosomes.

FIG. 23. Metaphase plate of second spermatocyte division showing seven chromosomes.

FIG. 24. Metaphase plate of second spermatocyte division showing six chromosomes.









## PLATE III.

FIG. 25. Anaphase of second spermatocyte division showing seven chromosomes going to each pole.

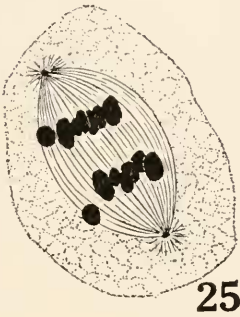
FIG. 26. Late anaphase of second spermatocyte division.

FIG. 27. Telophase of second spermatocyte division.

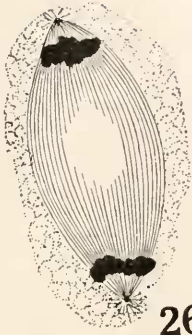
FIG. 28. Spermatids.

FIGS. 29 TO 31. Stages in the development of the spermatozoön.

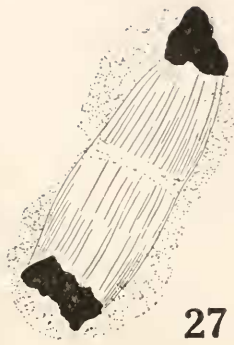
FIG. 32. Head and part of the tail of a spermatozoön.



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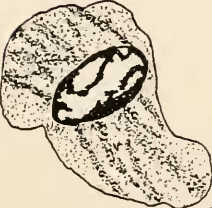
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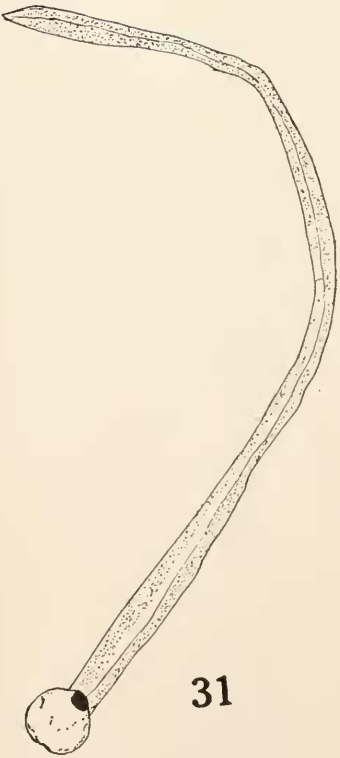
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# BIOLOGICAL BULLETIN

## NOTES ON THE BEHAVIOR OF THE ANT-LION WITH EMPHASIS ON THE FEEDING ACTIVITIES AND LETISIMULATION.

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### INTRODUCTION.

The ant-lion is one of the marvels of the insect world and is discussed in practically every text-book on entomology and in almost every popular book on insects. With the exception of results derived from attempts to analyze the behavior of these insects into tropisms (4), European papers may be epitomized as follows: (1) the pits are formed in sand that is protected from the weather; (2) the larva excavates this pit by moving backward in a constantly narrowing spiral and using its abdomen as a plowshare and its head for a shovel; (3) with one of its forelegs, the ant-lion scrapes the sand on to its head from the inner side of the spiral; (4) with its body entirely concealed, the larva lies in ambush, with its open jaws resting in the bottom of the finished pit; (5) by tossing up sand at random, the ant-lion forces insects that alight on the side of the pit to tumble to the bottom; (6) any small terrestrial invertebrate may become its prey; (7) there is no mouth opening, the food being imbibed through tubes formed by each mandible and another mouth-part.

In American scientific journals, I have been able to find only four articles treating of our ant-lion. The first and the longest of these is by Emerton (6). In the fall of 1870, he found a pit of *Myrmeleon immaculatus* De Geer, under the shade of a boulder, at Danvers, Mass. The larva was carried home and placed in a bowl of sand. Immediately it buried itself. After remaining beneath the surface for several days, it excavated a pit. No



mention is made of the larva using the foreleg to shovel sand on the flat head in the manner described by European and American popular writers. The larva was fed with flies. When given more than one at a time, it would kill all before eating any. It was kept over winter in a warm room. In May it spun its cocoon beneath the sand. In June the adult emerged, leaving half its pupa skin in the cocoon.

In August, 1871, Birge (2) found a colony of 600 ant-lions, under an overhanging cliff, in Albany County, N. Y. These pits were in a soil composed of fine disintegrated limestone commingled with pebbles and minute fragments of stone. Whenever an insect alighted upon the sides of the pit the ant-lion began to toss up the soil in all directions.

Moody (14) states that the ant-lion observed by him rests at the bottom of the pit with its jaws only showing, and that it throws up sand at escaping prey. It formed its cocoon June 4 and emerged July 8.

Moffat (13) writes: "Fine loose sand is evidently a necessity of their existence in any locality." He mentions the throwing up of sand when an ant steps on the side of the pit.

#### THE PIT.

Most accounts give the erroneous impression that the pits of ant-lions are formed only in sand. Even in a scientific magazine, Moffat (13) writes, "Fine loose sand is evidently a necessity of their existence in any locality." A loose friable soil protected, more or less, from rain and shielded from chickens and similar insect feeders is all that is needed. It may be anything from the finest dust to coarse sand. In open sheds with dirt floors, under porches where the place is not too dark, beneath low railroad bridges that span sandy, dusty, or cindery ground, under ledges of rock, and beneath the shelter of logs that do not touch the ground at all points are good places to look for them. From time to time, during the past three years, I have had, in my insectary, more than 500 ant-lions. Many of these were obtained in Kansas by my friend, Mr. Phil Rau; the remainder were collected in and about St. Louis. The majority of these were found in the loamy clay (loess) that forms much of the top

soil of Missouri and Kansas; some were found in cinders in sheltered places along railroad tracks; some, in disintegrated mortar along the walls of buildings; some, in the rotten-wood dust of hollow logs; none of these was obtained from sand. In the wide jelly glasses of my insectary, where each larva was kept in solitary confinement, some were placed in loamy clay, some in sifted coal ashes, some in coal ashes that had been weathered for a year, some in fine sand and others in coarse. The larvæ seemed to flourish as well in one medium as in the other.

The ant-lion usually begins the construction of its pit by striking out a circle in the friable earth. Using its abdomen as a plowshare and its head as a shovel, the larva burrows backward, in a circular path, just beneath the surface of the soil, tossing upward and outward the dirt that falls upon its head. Almost all of the articles that I have read state that this initial circle marks the outer boundary of the finished pit. With the American ant-lion of the Middle West this is not always so. In most cases observed by me the finished pit is wider than the diameter of this circle. In the first place, the falling inward of the soil as the excavation progresses enlarges the diameter. Then, too, the ant-lion sometimes enlarges the partly or apparently entirely completed pit. After this first circle is completed, within this the ant constructs, in a similar manner, a deeper adjacent circle and so on until the center is reached. Then, with the major portion of its body hidden in the walls of the pit and using its head and mandibles as a shovel, it tosses out the material from the bottom of the pit, until the dirt no longer runs down the sides.

European writers state that the ant-lion shovels sand on to its head by means of one of its forefeet, and Kirby and Spence (8) insist that, in excavating its burrow, the ant-lion reverses the direction it is going at the completion of each circle, so as to alternately exercise each foreleg. In our American ant-lion this pair of forelegs functions, not as a scrape, but as a brace to the body when the ant-lion is shovelling dirt or turning. Patient watching with a magnifying glass has failed to detect the fore-foot loading dirt upon the head; and certainly the ant-lion does not reverse the direction it is going every time it completes a

circle. The dirt gets upon the head by falling from above and from the sides, as the larva burrows backward through the soil. Some of this material comes from the outer edge as well as from the inner. While constructing its pit, the larva often pauses. After each rest it usually continues in the direction that it was going. On rare occasions, it does turn about and go in the opposite direction. This is usually when it has met some obstruction.

To test the matter more thoroughly, a sufficient portion of each of the forelegs of an ant-lion was amputated to render them much too short to be of value in shovelling soil on to the head. As soon as it was returned to its glass, the larva burrowed backward into the soil. For four days it remained beneath the surface. On that day it excavated a small pit. The next day the pit had been enlarged. On this day it was fed with ants (*Formica subsericea*). The ant-lion was then removed from the soil and examined under a simple microscope. The legs had not regenerated; each stump was covered with a ball of soil. This ant-lion had constructed its burrow without using its front feet as scrapers with which to load dirt on the head.

The force with which an ant-lion tosses the materials from its pit is astonishing. Often they are cast several inches beyond the rim. Sometimes the larva encounters particles which cannot be disposed of with a toss of the head. When these are not too heavy the insect has an unique method of disposing of them. The insect backs up the side of the pit with the obstacle poised on the posterior portion of its abdomen and deposits it beyond the edge of the pit. Although this behavior is described by Bingley (19), most writers do not mention it. Perhaps it sounds too much like a fairy tale; yet it is comparatively easy to induce an ant-lion to behave in this manner. I frequently induced it either by placing a small stone in the center of the ring of a pit that was being constructed; or, by depositing a similar object in the bottom of a completed pit. When the stone is placed in the center of the ring, as the ant burrows spirally inward, there is sure to come a time when the stone will fall into the furrow. When the ant-lion returns to that point it encounters the obstacle. Usually it burrows under the object and continues on part of

the way around the circle. Then, turning, it backs through the furrow thus made until it has inserted the tip of its abdomen under the impediment. It then backs slowly up the slope with the burden poised upon the tip of its abdomen. The edges of the abdominal somites and the stiff bristles thereon prevent the stone from slipping forward; while the dirt on each side prevents it from falling sidewise. Throughout this entire upward journey the whole body of the ant-lion is above the ground. It is an astonishing sight to see the insect backing, in almost a straight



Larval ant-lion. Dorsal view.



Larval ant-lion. Ventral view.

line, up the steep slope, with the burden poised on its back. When the burden has been disposed of, usually at the edge of the pit, the ant-lion turns about and returns to the bottom of the pit, usually in the furrow made by the upward struggle, and continues her digging. The furrows made before my eyes have always been straight or nearly so; but, one made in my absence was quite curved. When the object was placed in the bottom of a finished pit, sometimes the object was allowed to remain; but, in most cases, sooner or later, it would be removed, in the follow-

ing manner. When it had tossed up a few loads of dirt, the larva would back away from the obstacle in a straight or a curved line; then turning, it would back through the furrow thus made and proceed as described above. When the stone is too heavy for the insect to handle in the manner mentioned above, it either deepens the pit on one side of the obstacle, or buries the obstacle by mining under it, or else abandons the pit. In several important respects the behavior observed by me differs from that described by Bingley; (1) never once did I see the stone fall from the insect's back and roll to the bottom of the pit, (2) obstacles encountered in constructing the pit were usually removed at once, (3) such bodies were usually deposited just beyond the rim of the pit, (4) occasionally they were left on the side of the pit.

On rare occasions I have seen pits constructed in a different manner. Instead of beginning by striking out a circle, the ant-lion burrowed downward into the ground and began at once casting out the soil, thus making a pit of small diameter. Usually such pits were afterwards enlarged by burrowing into the walls and proceeding about as described above.

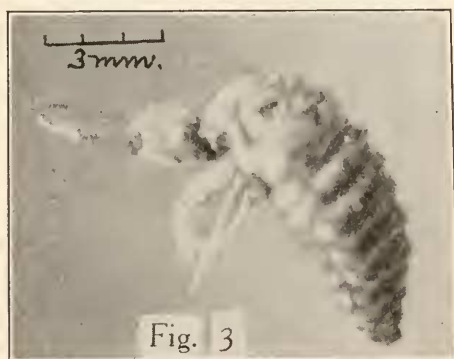
Thus my experience with the pit-building behavior of the ant-lion harmonizes with McCook's account (10); but is not in accord with that of Mrs. Comstock (5) who writes: "Wonderful stories are told about the way ant-lions dig their pits, marking out the outer margin in a circle and working inward. However, our common ant-lion of the East simply digs down into the sand and flips the sand out until it makes a pit."

The magnitude of the pit and the slope of the sides depend upon the size of the larva and the nature of the soil; the coarser the individual particles and the greater their specific gravity the more gentle the slope. In the loess about here the pits vary in diameter from less than half an inch to about two inches; the latter being the more abundant. Often the depth of the pit is almost as great as the diameter. Although a small ant-lion usually excavates a small pit, a small pit does not necessarily contain a small larva; for large larvæ sometimes construct small pits which they afterwards enlarge.

Occasionally one finds an isolated ant-lion pit; usually they occur in groups (Figs. 10, 11). In the same cluster the ant-lions

differ much in size, and this is true even in the early spring. Certain writers attribute these differences in size to the fact that some obtain more food than others. The following simple experiment lends support to this view. From a certain well-circumscribed area, containing about fifty ant-lion pits, a dozen larvæ were removed, on June 22, and placed in my insectary. A portion of these were well fed daily, the remainder were fed only occasionally. A few were lost by accidents. By August 8 all of the survivors of the well-fed lot had formed cocoons and a few imagoes had emerged. The poorly fed individuals were still larvæ. The majority of those left in the field were still larvæ.

Morphologically the ant-lion (Figs. 1-3) is well adapted to this pit-building behavior. The flat head, which, with the stout mandibles, forms an excellent shovel, is so articulated to the rest



Larval ant-lion. Lateral view.

of the body that it is possible to give it a powerful upward jerk. The abdomen is flat on the ventral and convex on the dorsal side, the whole tapering toward the tip in such a manner as to form an excellent burrowing instrument. From the sides of the body clusters of stiff bristles project outward and forward in such a manner that the body is prevented from slipping forward after it once has penetrated the earth. Then, too, the terminal claws on the legs (Fig. 12) make efficient anchors. The front of the dorsal portion of the prothorax is so rounded that dirt easily



falls forward and loads the shovel-like head. There is no functional anal opening; hence there is no danger of vigorous thrusts of the abdomen clogging the intestine with dirt.

#### FEEDING BEHAVIOR.

The finished pit is an inverted hollow cone, at the apex of which the wide-open mandibles of the larva, with their sharp teeth, await to grasp any unfortunate that happens to fall therein. What an efficient trap for small creeping invertebrates! The steep and unstable sides often cause the animals to fall to the bottom. If the intruder does not at once slide to the bottom, its struggles to escape tumble the soil upon the mandibles of the waiting ant-lion. Immediately the ant-lion begins to toss the soil upwards. The claim that the dirt is cast at the struggling creature is erroneous. Digging its mandibles edgewise into the bottom of the side of the pit, the ant-lion shovels out head-load after head-load of soil. It is not thrown at something; it is simply tossed upward and outward. Some of these random shots may take effect; and the constant undermining of the walls of the pit produces miniature landslides which, usually, drag the prey to the bottom of the trap.

Until something falls into the pit or alights on its treacherous sides, these mandibles of the larva usually rest horizontally in line with the body, which is hidden in the wall of the pit. Ordinarily the pits appear to be empty, for the mandibles are often covered with fine dirt. Even when the whole head is uncovered, its color harmonizes so perfectly with that of the soil as to render it invisible. As soon as its jaws close upon a creature the ant-lion backs deeper into the walls of the pit and, by interring its victim, subdues it. Thus the ant-lion is enabled to conquer creatures that are much larger and apparently stronger than it. Unless its first few struggles free it, the captive is doomed; for the ant-lion slowly but surely drags it deeper and deeper into the soil, while it feasts on its body juices. To assist in holding the prey while its body contents are being imbibed through the hollow mandibles, each mandible is provided, on its inner surface, with three stout teeth (Fig. 134).

MacLachlan (11), in discussing the feeding of the European

ant-lion (*Myrmeleon formicarius*) says: "The house flies and other small insects were usually dragged partially or wholly under the sand, whilst blue-bottles and similar bulky creatures were feasted upon on the surface." In his account of the feeding of the common ant-lion of the east, McCook remarks: "The ants were held off at 'arm's-length,' so to speak, and were thrashed and jerked about until they were exhausted. Meanwhile efforts at defence were made futile by the captor, who held its victim out of reach of any vital part." Neither of these accounts tallies exactly with my experience, although MacLachlan's can be harmonized with it. I have watched ant-lions feed thousands of times and have fed them with a variety of invertebrates. In every case the larva has attempted to drag its captive beneath the ground. In no case was the insect held off at arm's length



Empty cocoon of ant-lion.

as described by McCook. Often, a few moments after its capture, all that would be visible of an ant were the tips of its waving antennæ, or the extremity of its wriggling abdomen, or both. Naturally the captive struggled and squirmed; but there was no attempt on the part of the ant-lion to hold its prey at arm's length above the ground, while it thrashed it and jerked it. If the first closing of the mandibles does not capture the creature that



happens to fall into the pit, remaining at its post, the ant-lion elevates its head and makes repeated snaps at the creature as long as it remains near. It may be that the ocelli located at the base of the mandibles, on the dorsal side of the head, aid in this.

The name ant-lion is a misnomer; for it creates the impression that this insect feeds exclusively, or almost exclusively, upon ants. Such is not the case. Any small creeping invertebrate—be it insect, crustacean, or arachnid—is acceptable. Several of the most flourishing colonies of ant-lions found near St. Louis are located in the dirt floor of a dilapidated stone-crusher of an abandoned quarry. The diet of the inmates of those pits is composed largely of sow-bugs (*Porcellio*). Emerton (6) and MacLachlan (11) fed their ant-lions on living flies that had been disabled; Berce (1) reared his on living flies, wood-lice and earwigs. I supplied mine with



FIG. 5. Chrysalis of ant-lion that died on way to surface.

living specimens of the following invertebrates; caterpillars (even hairy ones), wood-lice, small roaches, small moths (held by the wings until the ant-lion had secured a hold), spiders, nymphal squash bugs, ants, small beetle larvæ, soft-bodied beetles, and bed-bugs. All of these were accepted and, after the juice had been sucked from each body, the dried remains were cast out of the pit.

The ant-lion has no mouth opening in the true sense of the word. The strong curved mandibles are perforated at the tip,

and along the ventral surface of each there runs a prominent tube through which the juices of the victim are sucked. This tube is composed of two parts. Along the whole of the ventral surface of each mandible [Fig. 13A] there is a deep groove with incurved edges. Another mouth part [Fig. 13B], probably the maxilla, fits so tightly into this groove of the mandible that, even when viewed with a  $\frac{2}{3}$ -inch objective, the two seem to form a single structure. With that power, on the underside of each mandible one sees two ridges. These mark the junctions of the two pieces; but, unless you had previously dissected a mandible, you would not suspect that there were two pieces and that they were not rigidly united. Turn the insect on its ventral surface,

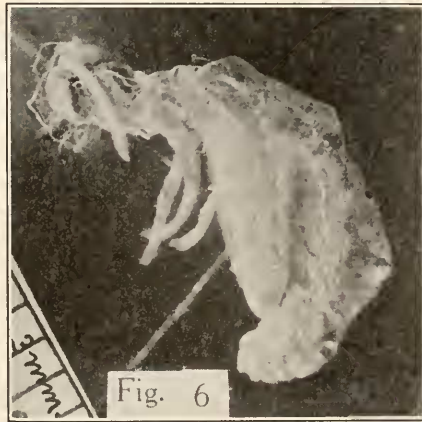


FIG. 6. Shed chrysalis skin of ant-lion.

carefully disarticulate the mandible, and, with a pair of dissecting needles, gently push it forward. Thus the other mouth part will be gradually drawn out of the mandible and left attached to the ventral part of the front of the head.

The ant-lion preys upon living invertebrates. How does it distinguish the living from the not-living? There may be several factors which help it solve this problem. The following experiments show that one attribute by means of which the ant-lion differentiates between desirable and undesirable prey is the exhibition of restlessness:

*Experiment 1.*—I fastened a bit of straw to the end of a silk thread.

*Twirling the other end of the thread between my thumb and fore-finger, I gently lowered it to the bottom of the pit. Three times in succession the ant-lion caught hold of the straw with its mandibles. Each time I jerked the string and thus removed the straw from its grasp.*

*Experiment 2.—I fastened a dead chinch bug to one end of a silk thread. Twirling the other end of the thread between my thumb and fore-finger, I gently lowered the bug to the bottom of the pit. Immediately the ant-lion seized it with its mandibles and held on until, by pulling on the thread, I had drawn the insect partly out of the pit. This experiment was tried with five different individuals. Four responded in the manner just described; the fifth made no response.*

*Experiment 3.—I fastened a piece of cotton to one end of a silk thread. Twirling the other end between my thumb and fore-finger, I gently lowered it to the bottom of the pit. The ant-lion gripped the bit of cotton with its mandibles and held on until I, by pulling on the thread, had dragged the larva partly out of the pit. This experiment was tried with five different individuals. The result was always the same.*

MacLachlan (11), in 1864, placed between two and three dozen ant-lions in a small box of sand and carried them from Fontainebleau, France, to London. When he arrived, about half of the larvæ had been killed and the juices of their bodies extracted by the others. In shipping ant-lions to me from Kansas, Mr. Rau placed a hundred or more in the same small box of dirt. In sorting over the material to place each one in an individual retainer, I always found several dead specimens that looked as though the juices of their bodies had been extracted. Are these deaths caused by cannibalism? To test the matter, an ant-lion was dropped into the pit of another individual. This experiment was repeated over and over again throughout a summer devoted largely to the field study of this creature. In the majority of cases the intruder escaped either by burrowing into the wall of the pit or else by backing out of it. In several instances, however, it became the prey of the rightful owner of the pit. Evidently, when opportunity permits, this creature is a cannibal.

## LOCOMOTION.

The forms of locomotion used in excavating pits and in removing obstacles therefrom have been described in the section on "The Pit" and will not be repeated here.

When placed on loose dry soil, the ant-lion may letisimulate. As soon as it begins to move, it burrows backward into the ground. If an ant-lion is placed in an open rectangular pasteboard box, it backs along, sometimes in a straight line and sometimes in a curved line, until it comes in contact with one of the sides. It then backs along that side until it comes to a corner, turning the corner it continues along to the next corner. It may continue thus for a long time, or it may vary it by creeping backwards up



FIG. 7. Cocoon of ant-lion, with chrysalis partly emerged. This cocoon is from a form that was raised in shifted coal ashes.

one of the angles until it reaches the top of the box and then pass downward to the ground. After it has once reached the side of the box, no matter how long it remains within the box, it almost never moves out into the open. These two simple experiments indicate that this insect is positively thigmotactic. With this statement must be coupled the reservation that, at times, the creature moves about with all of the upper portion of the body

exposed. This is the case when it is removing an obstacle from its pit.

Since this larva burrows downward into the earth, it may be considered positively geotactic; but, it must be remembered that it does not always pass downward. When disturbed in its pit, it usually backs upward, just beneath the surface, until the rim is reached; sometimes, it continues onward, in a horizontal direction, beneath the surface. MacLachlan (11) observed that, at night, they frequently make perigrinations over the surface of the ground. Then, too, they sometimes ascend vertical surfaces.

When placed on a horizontal surface [I used sheets of brass,

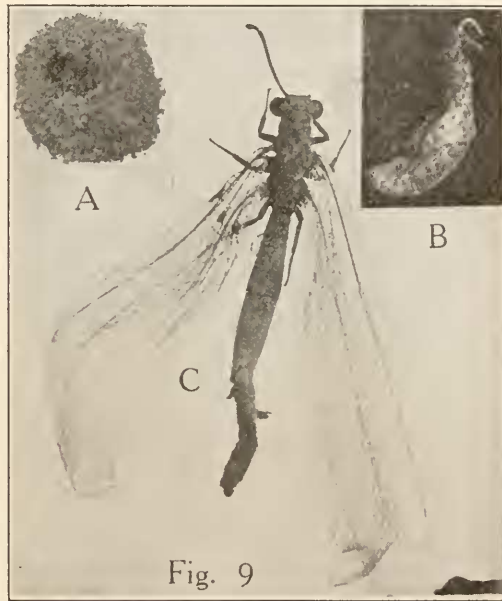


Ant-lion pit in one of my tumblers.

glass, wood, and cardboard], the larva backs away from the light. If placed with the tip of its abdomen toward the source of light, usually, it will move a short distance toward the light then turn, to either the right or the left, and back away in a straight line. This, coupled with the fact that, when placed on its back, the ant-lion invariably rights itself by turning away from the source of light, induces the conclusion that this creature is negatively phototactic; but, it must be remembered that in

constructing its burrow the larva crosses the light at every possible angle, and that, at times, it moves toward the light.

This insect invariably moves backward; never under any conditions does it move forward; yet it is capable of performing all the ambulatory feats possible to an insect that progresses in the orthodox way. It can move in a straight line, it can describe simple or s-shaped curves to either the right or the left, and it can ascend or descend rough surfaces inclined at any angle from zero to ninety degrees. By means of what structures does it perform these movements? Are they produced entirely by flexing the abdomen and trusting to the body bristles to prevent



Photograph showing the comparative size of cocoon, chrysalis and imago. They belong to the same ant-lion and are photographed to the same scale. A, cocoon; B, chrysalis skin; C, imago.

forward movements? Do the legs take any part in the movements? Do the mandibles assist? To answer these questions the following experiments were devised.

*Experiment 1.*—An ant-lion was placed on a glass plate arranged horizontally. By means of a hand magnifying glass every movement



*was watched.* It moved backward in jerks. The hind legs, which were doubled back under its abdomen, made jerky pulls. The middle pair of legs was directed outwards in almost a straight line. The anterior pair of legs was stretched forward. The tips of both the first and second pair of legs touched the glass. The mandibles took no part in the movement.

*Experiment 2.*—*The ant-lion was placed on the glass plate and held, in a horizontal position, above my head; so that I could look up at it with a magnifying glass.* The results were the same as in experiment 1; but it was easier to observe that the tips of all the legs touched the glass. The third pair of legs was the only pair making vigorous movements.

*Experiment 3.*—*I tilted the glass plate so that the posterior portion of the ant-lion was uphill.* When the angle became steep the ant-lion fell.

*Experiment 4.*—*Repeated number 1, substituting a pasteboard rectangle for the glass plate.* The result was the same as in experiment 1; but the insect moved faster.

*Experiment 5.*—*I tilted the paste-board rectangle so as to have the posterior portion of the insect up-hill.* Even when it had reached an angle of 90 degrees, the insect retained its hold. It moved upward, sidewise and downward.

*Experiment 6.*—*While the cardboard rectangle was inclined at a steep angle and the ant-lion was resting head downward, with a dissecting needle, I raised the tip of the abdomen from the support.* The ant-lion retained its hold.

These experiments show conclusively that the mandibles do not assist in locomotion; at the same time, they indicate that the hind pair of legs play an important rôle. Yet, so far as these experiments go, the hind legs might be mere grappling hooks to prevent the creature from slipping forward and the real locomotion be due entirely to the flexing and stretching of the abdomen, all forward motion being prevented by the stiff bristles on the sides of the body and the grip of the legs.

*Experiment 7.*—*A layer of dirt equal to the height of the greatest height of the ant-lion was spread on a glass plate. The ant-lion was placed on this pile of dirt.* The larva began to burrow backward into the dirt; but made practically no progress. By the

behavior of the body I could see that it was making vigorous movements with its third pair of legs; but it made practically no progress.

*Experiment 8.*—A layer of dirt equal in elevation to the greatest height of the ant-lion was placed on a pasteboard rectangle. An ant-lion was placed on this pile of dirt. Immediately it began to burrow backward and continued to progress at a rapid rate.

(In all of the experiments from 1-8 the same individual was used. The series was repeated many times with different in-



FIG. 10. A cluster of ant-lion pits (average cluster).

dividuals; but, in each case, the same individual was put through the eight experiments.)

In experiments 7 and 8 the presence of the dirt makes it necessary for more work to be performed in making progress backward. Since the height of the pile is the same in each case, the amount of work required is the same. Since the bristles are more numerous on the sides of the body than on the ventral surface, the presence of the dirt should give an added opportunity for them to function in preventing forward slipping of the body; hence, if the progress is due simply to a flexing and stretching of the body the ant-lion should be able to move just as fast, if not faster, on a glass plate with a layer of soil as on the naked glass. If, however, the hind legs play an important rôle in dragging the body backward, then the larva on the dirt-covered pasteboard should have a great advantage over the one on the dirt-covered glass plate. These



experiments prove, I think, that the hind legs assist in dragging the body backward. A microscopic examination of the legs reveals two terminal claws which function in this work (Fig. 12).

#### EMERGENCE OF THE IMAGO.

This section does not pretend to be a life history of the ant-lion. That the author hopes to make the subject of a future paper. This is simply an attempt to state some interesting facts about the last stages of the metamorphosis.

At the close of its larval period, the ant-lion constructs a subterranean spherical cocoon of silk and soil. In my insectary, most of the cocoons have been formed quite near the surface; sometimes projecting slightly above the soil. In one case, however, I found a cocoon on the bottom of the jelly glass,



FIG. 11. A cluster of ant-lion pits (small cluster).

fully two inches below the surface. In my insectary, the cocoons have been formed in July and in August and the imagoes have emerged in from 9-20 days thereafter; but I do not consider that I have sufficient data to warrant the statement that they are always formed at those times.

Inside of this cocoon the insect sheds its last larval skin and becomes a chrysalis. At the end of a certain period of time the anterior portion of the thorax protrudes from the cocoon (Fig. 7).

All of the accounts that I have read state that the chrysalis comes about half way out of the cocoon and from its dorsal

surface the imago emerges. In my limited experience I have noticed three methods of emergence. In one case the chrysalis protruded about half way out of the cocoon and the imago emerged from its back. In another case the chrysalis had left the cocoon entirely and protruded about half way out of the soil. In the third case both the head end and the tail end of the chrysalis remained within the cocoon and from its back the imago emerged. I am inclined to think the third case abnormal, caused by the head of the chrysalis becoming entangled in some strands of the cocoon. Fig. 6 is a photograph of the cast skin of that chrysalis, made just after I had removed it from its cocoon. It seems to me that the other two cases may be explained as follows: when

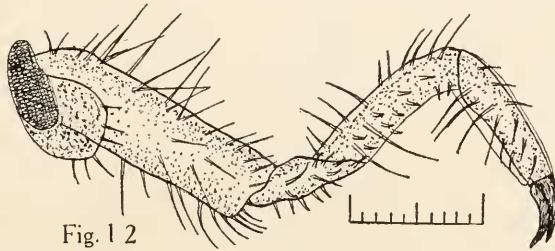


FIG. 12. One of the third pair of legs of an ant-lion larva.

the cocoon is near enough to the surface for the chrysalis to expose the upper portion of its body without coming entirely out of the cocoon it does so; when the cocoon is a little deeper then the chrysalis leaves the cocoon entirely and continues upward until the anterior portion of its body is above the surface.

When the cocoon is too far beneath the surface, the chrysalis dies on its upward journey. Fig. 5 is the photograph of such a chrysalis. It was found dead about half an inch below the surface. Attached to the bottom of the jelly-glass—about an inch below—the empty cocoon was found.

Soon after emerging the imago undergoes an enormous increase in size. It soon becomes more than twice as large as the chrysalis from which it came, and this without partaking of food. Fig. 9 illustrates this. The jelly glass containing the cocoon had been tightly closed to prevent the possible escape of the imago when it emerged. It emerged at an unexpected time and when discovered it was dead. It had lost one antenna and its body was

slightly damaged. Under the conditions it could not possibly have obtained food. Half exposed above the soil was the shed chrysalis skin, and a short distance below the surface the empty cocoon (Fig. 4) was found.

#### MISCELLANEOUS ACTIVITIES.

*Experiment 1.*—A ring of water eight inches in diameter was made on a glass plate and an ant-lion placed in the center of the dry space that the ring surrounded. The ring was one half of an inch in width. When the ant-lion reached the ring of water, it would usually turn and move away from it. Often, in turning, its mandibles would get into the water. In that case the mandibles would

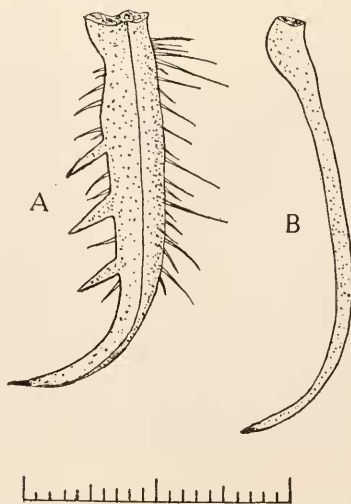


FIG. 13. The parts that form the sucking tube of the ant-lion larva. *A*, mandible. *B*, the part that fits into the groove of the mandible.

leave a broad water band wherever the creature went. After its mandibles had become wet, on its next approach to the water, it was apt to get some other part of its body wet. After that it was apt to move, away from the light, on through the water.

*Experiment 2.*—To see if there was anything about dirt as such that would direct an ant-lion to it, a pile of dirt three inches in diameter was placed in the center of a glass plate that was twelve inches

*square. The ant-lion was placed at various places near the periphery of the plate. Unless the ant-lion was placed in such a position that in going directly away from the light it would encounter the dirt, never once did an ant-lion discover it. Sometimes the larva passed within less than a centimeter of the dirt without being attracted by it.*

#### LETISIMULATION.

Letisimulation (from *letum*, death, and *simulare*, to feign) is a term coined by Weir<sup>1</sup> in 1899, to designate the death-like attitude assumed by individuals of many different groups of the animal kingdom, when roughly handled. While citing examples from among the worms, insects, reptiles, birds, and certain mammals, he leaves the impression that the most remarkable examples of death-feigning are to be found among the reptiles and certain mammals. Since that time much careful attention has been given to the letisimulation of insects. Barret (22) has studied it in the mole-cricket; Gee and Lathrop (26), and Johnson and Girault (32), in the plum curculio; Girault (27), in *trox*; Holmes (30), in the water scorpions; Newell (34) and Weiss (40), in the rice weevil; Riley (36), in dragon-fly nymphs; the Severins (37), in the giant water bugs, and Wodsedalek (41, 42), in May-fly nymphs and in a dermestid larva. In the light of the remarkable traits revealed by these investigators, were he writing his article today, Weir, no doubt, would agree with Homes that "it is among the insects that the death-feigning instinct reaches its highest development, occurring in a greater or less extent, in most of the orders. It is especially common in beetles and not unusual among bugs, but it is quite rare in the highest orders such as the Diptera, or flies, and the Hymenoptera, or ants, bees and their allies. It occurs in a few cases among the butterflies and moths, both in the imago as well as the larval state. The instinct is exhibited in different species in all stages of development from a momentary feint to the condition of intense vigor lasting for over an hour. Some insects may be severely mutilated, or, according to De Geer, even roasted over a fire before they cease feigning."

Although the activities of ant-lions have interested many

<sup>1</sup> Weir, "Dawn of Reason," pp. 202-214.

naturalists, very little attention has been paid to their death-feigning behavior. Emerton (6) asserts that rough handling caused his specimens to remain inactive for a time, and MacLachlan (11) states that the form observed by him letisimulates. Each of these students dismissed the matter with a single sentence.

The results recorded in this article are based on a careful laboratory study of 100 individuals selected at random; supplemented by observations made in the field. About 60 per cent. of these came from Kansas; the remainder from the vicinity of St. Louis, Mo. Some were quite small and others were almost large enough to form their cocoons. They were isolated in numbered jelly-glasses, partly filled with loamy loess, and were kept in an out-of-doors insectary, the north wall of which was constructed of wire netting. The other walls were opaque.

Any stimulus which produces a shock will usually cause an ant-lion to letisimulate. Rough handling, roughly turning it upon its back, dropping it from a slight elevation, all have a similar effect. I usually induced the feint by roughly turning the larva upon its back, or by dropping it from a slight elevation. Occasionally I found an individual that I could not induce to letisimulate; but this was a very rare occurrence.

Several investigators have thought it important to determine if the poses of letisimulating individuals are death attitudes. Based on a consideration of seventeen species of invertebrates, Holmes (31) concludes that the poses assumed were usually quite different from death attitudes; although there were some species in which they were always identical. I find that the ant-lion has not one, but several death attitudes; likewise it possesses a number of death-feigning postures, some of which resemble a death pose and some of which do not. The insect suddenly becomes rigidly immobile in whatever attitude it may be when it receives the shock. Absolute immobility is the character that is common to all cases; when the feint follows a long period of fasting, this inactivity often simulates death. The rigidity, however, is not so great as that described for certain insects. In some species of insects the rigidity of the parts during a death feint is so great that the insect may be picked up by a tarsus and



held out at right-angles without the leg bending in the least. That is not the case with the ant-lion. When an attempt is made to lift it, in that manner, by a tarsus, the leg bends and the insect awakes from its feint.

When an ant-lion is recovering from a feint, usually, although not always, there is a preliminary waving of the antennæ and a twitching of the legs and, sometimes, a movement of the head. Then the larva suddenly turns over. Throughout the whole series of experiments, a careful record was kept as to whether the insect turned towards the right or towards the left; towards the light or away from it. It was found that whether the insect turned toward the right or toward the left depended upon the location of the strongest light; for the ant-lion invariably turns away from the light.

Pinching the legs of a letisimulating individual almost always caused it to come out of its feint. Blowing upon a death-feigning larva would sometimes bring no response; at others it would induce a twitching of the legs; at yet others it would cause a complete recovery. Since the pinching of a leg, and even attempts to lift the letisimulating ant-lion by a leg, usually terminates the feint, I was surprised at the results produced by the following mutilations.

*Experiment 1.*—*With a pair of small, but sharp, dissecting scissors, I cut off the tip of a fore-leg of a letisimulating ant-lion.* The insect did not respond.

*Experiment 2.*—*With a pair of small, but sharp, dissecting scissors, I clipped off the tip of a mandible of a letisimulating ant-lion.* The insect did not respond.

*Experiment 3.*—*With a pair of small, but sharp, dissecting scissors, in rapid succession, I cut off the tips of both fore-legs and of a mandible of a letisimulating ant-lion.* The insect did not respond.

*Experiment 4.*—*With a pair of small, but sharp, scissors I severed the head from the body of a letisimulating ant-lion.* The insect did not respond, nor did it recover from the operation.

*Experiment 5.*—*A pair of small dissecting scissors, identical with those with which the above experiments were performed, was heated red hot, in a Bunsen flame, and allowed to cool.* This softened the

steel and made the edges quite dull. With these scissors an attempt was made to remove the tip of a leg of a letisimulating ant-lion. The scissors were too dull to cut through the chitin; instead of being severed, the leg became wedged in between the blades. The feint was terminated immediately.

With the exception of experiment number four, these amputations were performed several times. On one occasion, a larva recovered the moment I cut a leg; on another day, the same thing happened when I severed a mandible. With these two exceptions, the results were always as stated above. How shall we harmonize the fact that the pinching of a leg or, sometimes, the blowing of the breath on the larva terminates the feint, while the severing of a leg, or a mandible, or both invokes no response? Shall we decide that such a pinch produces a greater physiological shock than a sudden cut with a pair of sharp scissors? Is it possible that a breath of air produces a greater shock than an amputation with sharp instruments?

*Relative Duration of Successive Death Feints.*—In his study of the beetle *Scarites gigas* Fabre (24) found that the duration of the first five successive feints gradually increased from the first to the last. The Severins (37), in their study of the giant water bugs *Belostoma* and *Nepa*, and Gee and Lathrop (26), in their study of the plum curculio (*Conotrachelus nemuphar*), find great irregularity in the lengths of the successive feints.

To test the matter, an ant-lion was removed from its pit, placed on a board, and made to letisimulate by roughly turning it on its back. As soon as it recovered from one feint, it was roughly turned on its back and induced to letisimulate again. This was repeated until it had had an opportunity to letisimulate twenty times. By means of a stop-watch, the duration of each feint was obtained. One hundred individuals were thus experimented with and the results recorded in a table. Critically examined, the table revealed a number of interesting things. (1) There are marked individual variations. (2) In twenty opportunities the individual usually letisimulates less than twenty times. (3) The total time consumed in twenty opportunities to letisimulate varied from one minute to two hours and twenty-three minutes. The average for the 100 individuals was nineteen

and six-tenth minutes. (4) This death-feigning cannot be indefinitely prolonged. (5) The duration of the feints near the end of a long series of trials is always shorter than that of the earlier ones. (6) A curve representing the relative lengths of a series of letisimulations always contains two or more crests. (7) The longest feints usually occur somewhere near the beginning of the series. Of the 100 cases recorded, 33 letisimulated longest on the first trial, 11 on the second, 15 on the third, 2 on the fourth, 5 on the fifth, 7 on the sixth, 3 on the seventh, 5 on the eighth, 6 on the ninth, 5 on the tenth, 1 on the eleventh, 4 on the twelfth, 2 on the thirteenth, and 1 on the sixteenth. We have here, in a pronounced manner, the irregularity noticed by the Severins and Gee and Lathrop in the forms studied by them.

*Effects of Temperature upon the Duration of Letisimulations.*—To test this matter the 100 individuals mentioned were grouped according to the temperatures at which the experiments had been performed, and the results recorded in six tables. From the averages of those tables the following table was compiled.

TABLE SHOWING THE AVERAGES OF THE EFFECT OF TEMPERATURE ON THE LETISIMULATIONS.

Temperature in F. Degrees.	Number of Individuals Used.	Number of Letisimulations in Twenty Trials.	Maximum Time in Min. of a Single Letisimulation.	Number of the Trial on which Max. Let. was Made.	Total Time Consumed in Letisimulations.
60-65	7	15	3.35	4	14.19
65-70	12	16	6.86	2	26.11
70-75	17	17	7.25	5	35.11
75-80	23	13	3.97	5	13.06
80-90	17	12	5.91	6	13.30
85-90	21	9	3.83	6	12.15

If we were to rely upon these averages, we would conclude that up to 75° F. both the length of the maximum feint and the total duration of twenty feints vary directly with the temperature; and that beyond that point there is no definite relation between temperature and the feints. This conclusion, however, is not supported by a critical study of the individual records from which the averages were compiled. To test the matter further, four individuals were selected and each put through five series of twenty letisimulations, each series being conducted at a different temperature. The results were recorded in four tables. There



was no obvious relation between temperature and the duration of the feints.

*Effects of the Strength of Stimulus upon Letisimulation.*—To get the stimulus as nearly uniform as possible, the ant-lion was gently shoved from a glass ledge and caused to fall three inches. To secure a strong stimulus the ant-lion was permitted to fall upon a glass plate; to secure a weak one it was allowed to drop on a layer of cotton batting. The results of experimenting with 100 ant-lions was tabulated. In 36 cases the first letisimulation following a strong stimulus was the longer and in 58 cases the first feint following a weak stimulus was the longer. In six cases the duration of the feint was the same for each stimulus. The average of 100 individuals gave the duration of the first letisimulation following a weak stimulus as of longer duration than the first following a strong stimulus. These data do not seem to warrant a conclusion.

*Effects of Hunger upon Letisimulation.*—Certain selected individuals were well fed and others were forced to fast for a long time before they were used for experiments identical with those mentioned above. The results were carefully tabulated. No relation could be detected between hunger and the length of the letisimulation.

Apparently the reason for the longest letisimulation being located sometimes at one place and sometimes at another in the series is due to some internal (physiological) factor not revealed by these experiments.

Weir<sup>1</sup> considers the letisimulation of animals "one of the greatest evidences of intellectual action, on their part." Hamilton (29), Webster (39) and a few others feel that the creatures consciously fear death and take this means to avoid it. Dr. Lindsley, in "Mind in Animals," thinks "this must require great command in those that practice it." However, the majority of modern students of the subject look upon it as merely a remarkable instinct.

No one who is acquainted with how slowly the ant-lion recovers from injuries could, for a moment, consider anything intellectual, which induces it to passively submit to portions of its legs and of

<sup>1</sup>Weir, "Dawn of Reason," 1889, p. 202.

its mandibles being amputated. The tonic contraction of the muscles and the diminished reflex irritability suggest hypnotic phenomena and lead one to agree with Holmes (31) that "the instinct of feigning death is doubtless connected with much of what has been called hypnotism in the lower animals." It is well known that most animals pause momentarily when confronted with an unexpected or violent stimulus. To me the letisimulation of the ant-lion appears to be such a pause prolonged and exaggerated. The more I ponder over the results of my experiments upon the death-feigning of the ant-lion, the more I feel inclined to exclaim with James: "It really is no feigning of death at all and requires no self-command. It is simply terror paralysis which has been so useful as to become hereditary."

#### CONCLUSIONS.

1. The pits of the ant-lion are not confined to sand; they may be found in any kind of dry friable soil, that is protected from the rain and from insect-eating creatures. They are usually in clusters; but, occasionally, a solitary pit is found. On yet rarer occasions, pits may be found that are not under a shelter.

2. The ant-lion of the Middle West has two methods of excavating its pits. Usually it furrows backward, excavating a series of concentric, adjacent circles, each deeper than the last, and shovelling out the soil with its head. The front of the body is so curved as to make it easy for the dirt to fall forward on the head. In the second method, the larva simply burrows downward into the ground and tosses out the soil with its head until the sides of the pit become approximately stable. Pits formed by the second method are usually subsequently enlarged.

3. The ant-lion removes a medium sized obstacle from its pit by inserting the tip of its abdomen under it and, with the burden poised on its abdomen, backing slowly up the slope.

4. After its trap has been completed, the ant-lion rests quietly, in practically a horizontal position, with its body beneath the soil and its open mandibles in the bottom of the pit.

5. Any invertebrate, be it insect, arachnid, or crustacean, that happens to fall into the trap is acceptable food. Some escape, but the larva attempts to capture all. When captured, the

victim is dragged partly or wholly beneath the soil, and the juices imbibed through the hollow mandibles. Later the dried carcass is tossed away.

6. The ant-lion may be considered positively geotactic, positively thigmotactic and negatively phototactic; with the reservation that all of its movements cannot be explained as tropisms in the Loebian sense.

7. It is impossible for the ant-lion to move forward; but, in its backward movements, it can move in straight lines or curves, and can scale vertical surfaces that are not too smooth. The hind legs assist in producing this backward movement, and the other legs brace the body.

8. Sometimes it avoids water and at others it backs into it.

9. If the spherical cocoon of this insect is near the surface of the ground, the chrysalis comes only part of the way out and the imago emerges from its back. If the cocoon is at a slightly greater depth, the chrysalis comes entirely out of the cocoon and part of the way out of the ground. If the cocoon is at a greater depth, the chrysalis emerges entirely from the cocoon and perishes on the way to the surface of the ground.

10. Rough handling or dropping from a slight elevation will usually cause an ant-lion to letisimulate. The length of a feint and the position of the longest feint in a series of successive feints varies in different individuals and in the same individual at different times.

11. There is no obvious relation between the temperature, the strength of the stimulus, or fasting and the duration of a letisimulation.

12. If the relative durations of the successive feints of a long series of letisimulations are plotted, the curve will have two or more crests.

13. In the ant-lion all letisimulation poses are not death attitudes. The ant-lion has no characteristic death-feigning posture. It is to be grouped with those insects in which the letisimulation pose varies with the attitude of the individual at the time when the stimulating shock is received.

14. Although pinching a leg and, sometimes, even blowing on the body, will usually cause a letisimulating ant-lion to come out

of its feint, in the majority of the cases, it will submit to the clipping off of the tips of its legs and of its mandibles without responding in any visible manner.

15. In the ant-lion letisimulation seems to be but an exaggerated prolongation of the pause made by most animals when they are startled. The total behavior of a death-feigning ant-lion supports Holmes's contention that "the instinct of feigning death is connected with much of what is called hypnotism in the lower animals"; and endorses James, when he says: "It is really no feigning of death at all and requires no self-command. It is simply terror paralysis which has become so useful as to become hereditary."

## REFERENCES

BEHAVIOR OF ANT-LIONS<sup>1</sup>

1. Berce.  
'65 [Records his experience in rearing *Myrmeleon formicarius* from the larva.] Bull. Soc. Entom. Fr., p. xlvii.
2. Birge, E. A.  
'73 The Ant-lion. Amer. Nat., Vol. VII., p. 432.
3. Brischke, C.  
'79 Ueber das Eierlagen von *Myrmeleon*. Ent. Nachr., pp. 29-30.
4. Comes, Salvatore.  
'09 Stereotropismo, Geotropismo, e Termotropismo nella Larva de *Myrmeleon formicarius* L. Atti della Accademia Gioenia di Scienze Naturali in Catania. Anno LXXXVI, memoria IV, pp. 1-14.
5. Comstock, Mrs. A. B.  
'13 Handbook of Nature Study, pp. 395-397.
6. Emerton, J. H. The Ant Lion. Amer. Nat., 1871, Vol. IV., pp. 705-708.
7. Ferrari.  
'65 Notes on the Habits of the Larva of *Acanthaclisis*. Wien Ent. Mon., Bd. VIII., p. 107.
8. Kirby and Spence.  
'56 Introduction to Entomology, 7th edition, 242-244.
9. Lydekker, R.  
New Natural History, Vol. VI., pp. 165-166.
10. McCook, H. C.  
'07 Nature's Craftsmen, pp. 129-139.
11. MacLachlan.  
'65 Habits of *Myrmeleon formicarius* L. Ent. Mag., Vol. II, pp. 73-75.
12. MacLachlan.  
'66 [Note on eggs.] Bull. Soc. Entom. Fr., xvi.
13. Moffat, J. A.  
'84 Notes on Ant Lions. Canad. Entom., Vol. XVI., pp. 121-122.

<sup>1</sup> Practically every text-book on entomology and many popular books on insects treat of ant-lions. With the exception of those to which special mention is made, they are omitted from this list.

14. **Moody, H. L.**  
'73 Habits of *Myrmeleon immaculatus*. Canad. Entom., Vol. V., pp. 63-65.
15. **Navás, P.**  
'00 Algunas Costumbres de las Hormigas y Hormigaleones. Act. Soc. Espan., pp. 219-222.
16. **Navás, L.**  
'13 Biologische Beobachtungen. Ent. Mitt. Berlin, Bd. II., s. 81-87.
17. **Navás, P. J.**  
'12 Notes Sobre Mirmeleonidos. Broteria, Vol. X., pp. 29-75.
18. **Redtenbacher, J.**  
'84 Uebersicht der Myrmeleoniden Larven. Denk. Ak. Wien, Bd. XLVIII., s. 335-368, pls. I-VII. [Gives a bibliography up to 1883.]
19. **Romanes.**  
'83 Animal Intelligence, pp. 235-236.
20. **Rudow, F.**  
'78 *Myrmeleon formicalynx* Pupating without Forming a Cocoon. Ent. Nachr., Bd. IV., s. 272.
21. **Step, Edward.** Marvels of Insect Life, pp. 108-113.

## LETISIMULATION OF INSECTS.

22. **Barret, O. W.**  
'02 The Changa, or Mole Cricket (*Scaptericus didactylus* Latrl.) in Porto Rico. Porto Rico Agri. Exp. Sta. Bull.
23. **DeGeer, C.**  
'74 Memoirs pour Servir a l'Histoire des Insectes, Vol. IV., pp. 229.
24. **Fabre, J. H.**  
Souvenirs Entomologiques, 7th series, pp. 14-27.
25. **Fabre, J. H.**  
'98 La Simulation de la Mort. Rev. Quest. Sci., Vol. XIV., pp. 111-139.
26. **Gee and Lathrop.**  
'12 Death Feigning of *Conotracheus nemuphar* Herbst. Ann. Ent. Soc. of Amer., Vol. V., pp. 391-399.
27. **Girault, A. A.**  
'13 Fragments of North American Insects. Entom. News, 1913, Vol. XXIV., pp. 338-344.
28. **Grote.**  
'88 Insects Feigning Death. Canad. Entom., Vol. XX., p. 120.
29. **Hamilton.**  
'88 Insects Feigning Death. Canad. Entom., Vol. XX., p. 179.
30. **Holmes, S. J.**  
'06 Death Feigning in *Ranatra*. Journ. Comp. Neur. and Psychol., Vol. XV., pp. 305-349.
31. **Holmes, S. J.**  
'08 The Instinct of Feigning Death. Pop. Sci. Monthly, 1908, Vol. LXXII., pp. 179-185.
32. **Johnson and Girault.**  
'06 The Plum Curculio. Circ. 73 Bu. of Entom. U. S. Dept. of Agri.
33. **Kirby and Spence.**  
'56 An Introduction to Entomology, 7th edition, p. 412-413.

34. **Newell, W.**  
    '13 Notes on the Rice Weevil and Its Control. Jour. Ec. Entom., Vol. VI., pp. 55-61.
35. **Piéron, H.**  
    '04 L'Immobilité Protectrice chez les Animaux. Revue Sci., Vol. LXXIII., pp. 523-527.
36. **Riley, C. F. Curtis.**  
    '12 Observations on the Ecology of Dragon-Fly Nymphs. Ann. Entom. Soc. of Amer., Vol. V., pp. 273-292.
37. **Severin, H. H. P. & H. C.**  
    '11 Habits of *Belostoma (Zaitha) flumineum* Say and *Nepa apiculata* Uhler, with Observations on Other Closely Related Aquatic Hemiptera. Jour. N. Y. Entom. Soc., Vol. XIX., pp. 99-108.
38. **Slater, C. R.**  
    '79 Simulation of Death by Coleoptera and Arachnida. Sci. Gos., Vol. XV., pp. 160-161.
39. **Webster.**  
    Insects Feigning Death. Canad. Entom., 1888, Vol. XX., p. 199.
40. **Weiss, H. B.**  
    '13 Notes on the Death Feint of *Calandra orysæ*. Canad. Entom., pp. 135-137.
41. **Wodsdalek, J. E.**  
    '12 Natural History and General Behavior of the Ephemeridæ Nymphs *Heptagenia interpunctata* Say. Ann. Entom. Soc. of Amer., Vol. V., pp. 31-40.
42. **Wodsdalek, J. F.**  
    '12 Life History of Habits of *Trogoderma tarsale* (Melsh). Ann. Entom. Soc. of Amer., Vol. V., pp. 367-381.
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# THE EFFECT OF CERTAIN ORGANIC AND INORGANIC SUBSTANCES UPON LIGHT PRODUCTION BY LUMINOUS BACTERIA.

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While engaged in a study of the chemistry of light production by luminous bacteria I had occasion to investigate the effect of diluting the sea water with distilled water and with isotonic sugar solution and the influence of the various salts of sea water, of acids and alkalies, and of certain anæsthetics upon the emission of light. The results are of interest for comparison with the known effects of these substances on other organisms and with other vital manifestations of life.

In all experiments, except where otherwise noted, one drop of the dense emulsion of luminous bacteria (a form isolated from squid at Woods Hole, Mass.) was added to 30 c.c. of solution in an uncorked Erlenmeyer flask and the whole thoroughly mixed. For comparative observations it is essential that the eye be thoroughly adapted to the dark and that each flask be oxygenated by shaking, before judging as to the emission or absence of light. Observations were made after 10 minutes, one hour and 24 hours.

TABLE I.  
EFFECT OF DILUTION OF SEA WATER WITH WATER AND WITH *m* CANE SUGAR SOLUTION.

Dilution with Water.					Dilution with <i>m</i> Cane Sugar.				
Parts Sea Water.	Parts Water.	Light after			Parts Sea Water.	Parts <i>m</i> Sugar.	Light after		
		10 Min.	1 Hr.	24 Hrs.			10 Min.	1 Hr.	24 Hrs.
2	1	+	+	+	2	1	+	+	+
1	1	faint	faint	—	1	1	+	+	+
1	2	faint	very faint	—	1	2	+	+	+
1	4	faint	—	—	1	4	+	+	faint
1	6	very faint	—	—	1	6	+	+	faint
1	10	—	—	—	1	10	+	+	faint
1	14	—	—	—	1	14	+	+	—
1	20	—	—	—	1	20	+	+	—
Sea water		+	+	+	<i>m</i> cane sugar		+	+	—
Water		—	—	—					



It will be noted from the above Table I. that the bacteria cease to give off light and experiment shows that they are killed by too great dilution with water. That this effect is not entirely due to the absence of salt but is chiefly due to a cytolysis through lowered osmotic pressure is shown by diluting the sea water with an inert isotonic solution, cane sugar. Some salt is necessary for the continued production of light as the bacteria no longer glow after twenty four hours' emersion in *m*-sugar, a fact of no great surprise as unicellular freshwater luminous animals are unknown.

TABLE II.  
EFFECT OF ACID AND ALKALI.

Conc. of Acid and Alkali Added to Mg-free Sea Water, <i>m</i> /2 (100 NaCl+2.2 KCl+2CaCl <sub>2</sub> ) in Syracuse Watch Glasses.	Light after		
	10 Min.	1 Hr.	24 Hrs.
<i>n</i> /2000 HCl.....	—	—	—
<i>n</i> /4000 HCl.....	faint	—	—
<i>n</i> /8000 HCl.....	+	faint	—
<i>n</i> /16000 HCl.....	+	+	—
<i>n</i> /32000 HCl.....	+	+	faint
<i>n</i> /500 valerianic acid.....	—	—	—
<i>n</i> /1000 " ".....	faint	—	—
<i>n</i> /2000 " ".....	faint	—	—
<i>n</i> /4000 " ".....	+	faint	—
<i>n</i> /8000 " ".....	+	+	—
<i>n</i> /16000 " ".....	+	+	faint
<i>n</i> /500 NaOH.....	—	—	—
<i>n</i> /1000 NaOH.....	—	—	faint <sup>1</sup>
<i>n</i> /2000 NaOH.....	+	+	+
<i>n</i> /250 methyl amine.....	—	—	—
<i>n</i> /500 " ".....	faint	—	faint <sup>1</sup>
<i>n</i> /1000 " ".....	faint	+ <sup>1</sup>	faint
<i>n</i> /2000 " ".....	+	+	+
Mg-free sea water.....	+	+	faint
Sea water.....	+	+	+

<sup>1</sup> Probably due to neutralization of alkali through absorption of CO<sub>2</sub>.

As was to be expected acids and alkalis prevent light emission in very weak concentration, the acids in much weaker concentration than the alkalis. In fact the bacteria are very sensitive to acid and will not even phosphoresce with any brilliancy in a neutral medium.

The organic acid (valerianic) and alkali (methyl amine) have less effect than the inorganic, a result at variance with my results for other organisms which are usually affected more readily by the weak than by the strong acids and alkalis.<sup>1</sup>

<sup>1</sup> Harvey, E. N., "Studies on Acids," in Carnegie Institution Publications No. 212, p. 143, 1915; on alkalis, *id.*, No. 183, p. 131, 1914.



TABLE III.  
EFFECT OF VARIOUS COMBINATIONS OF THE SALTS OF SEA WATER.

Salt Combinations.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Sea water.....	+	+	+
Artificial Sea Water, $m/2$ (100 NaCl+2.2 KCl+ 2 $\text{CaCl}_2$ +10 $\text{MgCl}_2$ )+ $n/4000$ NaOH.....	+	+	+
Neutral artificial sea water.....	+	+	faint
$m/2$ NaCl.....	+	+	faint
$m/2$ KCl.....	+	faint	—
$m/3$ $\text{CaCl}_2$ .....	—	—	—
$m/3$ $\text{MgCl}_2$ .....	—	—	—
$m/2$ (100 NaCl+2.2 KCl).....	+	+	faint
$m/2$ (100 NaCl+2 $\text{CaCl}_2$ ).....	+	+	faint
$m/2$ (100 NaCl+10 $\text{MgCl}_2$ ).....	+	+	faint
$m/2$ (100 NaCl+2.2 KCl+2 $\text{CaCl}_2$ ).....	+	+	faint
$m/2$ (100 NaCl+2.2 KCl+10 $\text{MgCl}_2$ ).....	+	+	faint
$m/2$ (100 NaCl+2 $\text{CaCl}_2$ +10 $\text{MgCl}_2$ ).....	+	+	faint

The most interesting point brought out in the above table is the independence of these bacteria of a balanced medium. The bacteria live and phosphoresce in pure NaCl without the addition of any bivalent ions. This is true even when the solution is changed three times to remove the last traces of Ca in the bacteria. KCl is also relatively non-toxic, although more so than NaCl.  $\text{CaCl}_2$  and  $\text{MgCl}_2$  are very toxic when alone. All combinations of NaCl with the other ions of sea water sustain the bacteria well except that they are neutral media and hence the phosphorescence is dimmed after 24 hours. That pure NaCl should have so little effect on light production is astonishing when we consider its poisonous effect on other marine organisms and tissues, particularly on ciliated cells.

The effect of the alcohols (Table IV.) on light production is very similar to their effect on other life processes: they exert an inhibiting or anæsthetic action which is perfectly reversible. If alcohol solutions containing bacteria which have stopped emitting light are diluted with sea water, light production again begins. As with other tissues the higher the alcohol in the series the greater anæsthetic power it has.

The effect of a number of other substances was studied in a very rough way—namely, by adding a small quantity of the substance to a sea water emulsion of the bacteria in test tubes and then shaking the tubes. With toluol, benzol, ether, chloroform, carbon disulphide, carbon tetrachloride and ethyl butyrate

TABLE IV.  
EFFECT OF ALCOHOLS.

Conc. of Alcohol Added to Sea Water.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Methyl alcohol, 2 <i>m</i> .....	—	—	—
HCH <sub>2</sub> OH, 1.5 <i>m</i> .....	+	very faint	—
HCH <sub>2</sub> OH, <i>m</i> .....	+	faint	—
HCH <sub>2</sub> OH, <i>m</i> /2.....	+	+	+
HCH <sub>2</sub> OH, <i>m</i> /3.....	+	+	+
Ethyl alcohol, <i>m</i> .....	—	—	—
CH <sub>3</sub> CH <sub>2</sub> OH, <i>m</i> /1.5.....	very faint	—	—
CH <sub>3</sub> CH <sub>2</sub> OH, <i>m</i> /2.....	+	faint	faint
CH <sub>3</sub> CH <sub>2</sub> OH, <i>m</i> /3.....	+	+	+
Propyl alcohol, <i>m</i> /3.....	—	—	—
CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> OH, <i>m</i> /4.....	very faint	—	—
CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> OH, <i>m</i> /6.....	faint	very faint	—
CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> OH, <i>m</i> /8.....	+	faint	—
CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> OH, <i>m</i> /15.....	+	+	+
Isobutyl alcohol, <i>m</i> /10.....	—	—	—
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> OH, <i>n</i> /12.....	very faint	—	—
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> OH, <i>m</i> /16.....	+	very faint	—
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> OH, <i>m</i> /20.....	+	+	—
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> OH, <i>m</i> /24.....	+	+	+
Amyl alcohol, <i>m</i> /20.....	—	—	—
C <sub>2</sub> H <sub>5</sub> CH <sub>3</sub> CHCH <sub>2</sub> OH, <i>m</i> /40.....	—	very faint <sup>1</sup>	+ <sup>1</sup>
C <sub>2</sub> H <sub>5</sub> CH <sub>3</sub> CHCH <sub>2</sub> OH, <i>m</i> /80.....	very faint	very faint	+ <sup>1</sup>
C <sub>2</sub> H <sub>5</sub> CH <sub>3</sub> CHCH <sub>2</sub> OH, <i>m</i> /160.....	faint	+ <sup>1</sup>	+
C <sub>2</sub> H <sub>5</sub> CH <sub>3</sub> CHCH <sub>2</sub> OH, <i>m</i> /320.....	+	+	+
Capryl alcohol, <i>m</i> /400.....	—	—	—
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>6</sub> CH <sub>2</sub> OH, <i>m</i> /800.....	very faint	very faint	—
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>6</sub> CH <sub>2</sub> OH, <i>m</i> /1600.....	faint	faint	—
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>6</sub> CH <sub>2</sub> OH, <i>m</i> /3200.....	faint	faint	+ <sup>1</sup>
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>6</sub> CH <sub>2</sub> OH, <i>m</i> /6400.....	+	+	+
Sea water.....	+	+	+

<sup>1</sup> Probably due to evaporation of alcohol.

the light was found to disappear almost immediately; with tannin, chloral hydrate, vanillin and sodium glycocholate the light had disappeared in the course of one hour while saponine, amygdalin, and sodium taurocholate had no effect. It is surprising that saponin has no effect on luminous bacteria when we consider its great cytolytic power on other forms in very small concentration.

#### SUMMARY.

The effects on luminous bacteria of dilution of sea water with water and *m* sugar solution; of HCl and valerianic acid; of NaOH and methyl amine; of the salts of sea water in different combinations; and of methyl, ethyl, propyl, butyl, amyl and capryl alcohol were studied. The points of interest in the results are indicated after each table.

## FURTHER NOTES ON THE CHROMOSOMES OF THE CERCOPIDÆ.

ALICE M. BORING AND RAYMOND H. FOGLER.

The chromosomes in the spermatogenesis of five species of this family of Hemiptera have already been studied by Stevens<sup>1</sup> and Boring.<sup>2</sup> Three more species have now been studied in comparison with those previously studied. They are *Philaenus lineatus*, *Aphrophora parallela* and *Clastoptera proteus*. Each of these three species belongs to a genus in which one or more species has already been studied, so this gives a chance to compare the spermatogenesis in closely related species. This has been done very carefully by McClung<sup>3</sup> for some families of Orthoptera. The entire family Acrididæ has the same spermatogonial chromosome number, 23, and the Locustidæ has 33, but within each family there are generic and specific cytological differences. The family Cercopidæ of the Hemiptera does not show as closely graded a series of cytological differences as the orthopteran families studied by McClung. The facts as found are here recorded.

The material was collected at Woods Hole<sup>4</sup> and in Orono; *Philaenus lineatus* from grasses, *Aphrophora parallela* from Scotch pines, and *Clastoptera proteus* from alders. Dr. Herbert Osborn has very kindly identified the species of the material. Flemming's and Gilson's solutions were used for fixation, and iron hæmatoxylin for staining.

*Philaenus lineatus* has 29 chromosomes as spermatogonial number (Fig. 1), two of which are larger than the others. The odd chromosome is round or oval in the early (Fig. 2) as well as

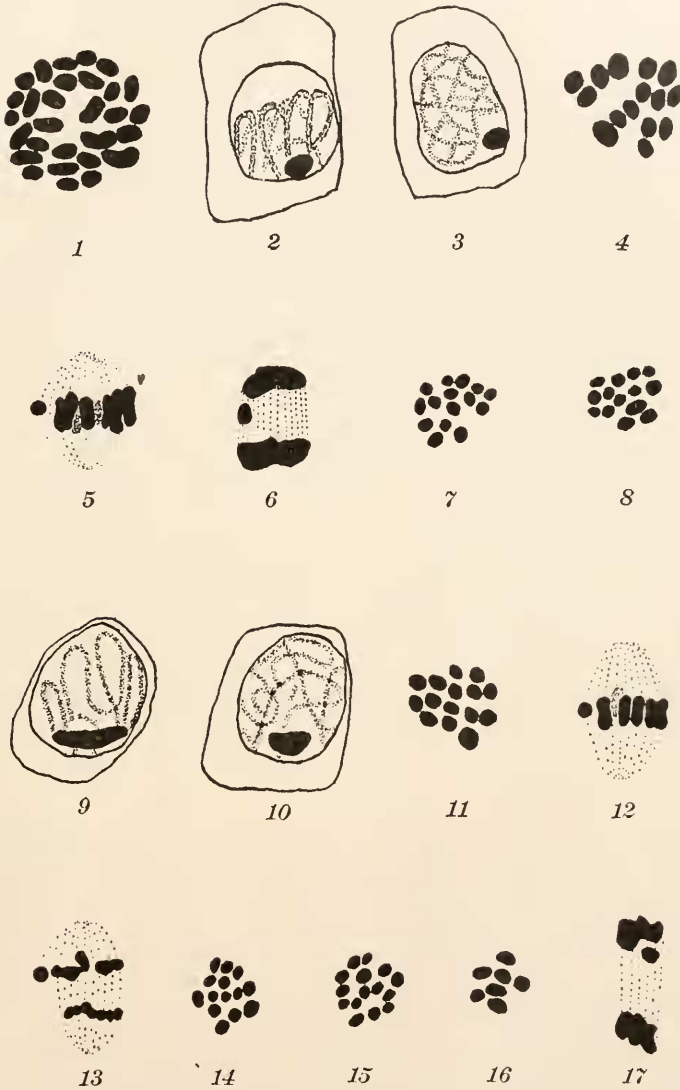
<sup>1</sup> N. M. Stevens, '06, "Studies in Spermatogenesis," Pt. II., Carnegie Institute, Washington.

<sup>2</sup> A. M. Boring, '07, "A Study of the Spermatogenesis in the Membracidæ," etc., *Jour. Exp. Zool.*, 4, p. 469. '13, "The Chromosomes of the Cercopidæ," *Biol. BULL.*, 24, p. 133.

<sup>3</sup> C. E. McClung, '08, "Cytology and Taxonomy," *Kans. Univ. Bull.* 4.

<sup>4</sup> We wish to thank the Director of the Marine Biological Laboratory for the privileges of the laboratory during the summers of 1913 and 1915, at which time this material was collected.

the late (Fig. 3) spireme stages. The reduced number of chromosomes is 15 in the first spermatocytes, one of which is larger than the others (Fig. 4). The odd chromosome is univalent



(Fig. 5) and does not divide in the first spermatocyte division (Fig. 6). The second spermatocytes have partly 15 (Fig. 7)

and partly 14 (Fig. 8) chromosomes. The chromosome number is specific, as the reduced number is 15, while only 12 are found in *Philaenus spumarius*. But the roundness of the odd chromosome throughout the spireme stages is a feature common to both species of this genus, and distinguishing it from the species of the genus *Aphrophora*.

*Aphrophora parallela* has 15 chromosomes as reduced number, with one largest chromosome (Fig. 11). The odd chromosome is elongated in the early spireme stages (Fig. 9) and becomes more nearly round in the later stages (Fig. 10). The odd chromosome is, as usual, univalent (Fig. 12) and does not divide in the first spermatocyte division (Fig. 13). The chromosome number in the second spermatocytes is 14 and 15 (Figs. 14 and 15). Again in this species, the chromosome number is different from that in the other species of the same genus, that is, 15, in comparison with 14 in *Aphrophora quadrinotata* and 12 in *Aphrophora spumaria*. The long odd chromosome in the early spireme stages is a common feature of both *A. spumarius* and *A. parallela*, and distinguishes them from the genera *Philaenus* and *Clastoptera*. The early spireme stages of *A. quadrinotata* were not studied. The species formerly classified as *A. quadrangularis* has since been put into the genus *Lepyronia*. This species does not possess the long odd chromosome characteristic of the genus *Aphrophora*.

*Clastoptera proteus* has 7 as reduced chromosome number (Fig. 16), one less than the reduced number in *Clastoptera obtusa*. Unfortunately only a few stages were found in this material, so

TABLE I.

Genus.	Species.	Reduced Chromosome Number.
<i>Philaenus</i>	<i>spumarius</i> .....	12
"	<i>lineatus</i> .....	15
<i>Aphrophora</i>	<i>spumaria</i> .....	12
"	<i>quadrinotata</i> .....	14
"	<i>parallela</i> .....	15
<i>Lepyronia</i>	<i>quadrangularis</i> .....	11
<i>Clastoptera</i>	<i>proteus</i> .....	7
"	<i>obtusa</i> .....	8

that the only other significant point that was observed was that the first spermatocyte division is the one in which the odd chromosome does not divide (Fig. 17) as in all the species of this family.

The eight species of *Cercopidæ* in which the spermatogenesis has so far been studied belong to four genera. The chromosome number (reduced) varies from 7 to 15. The chromosome number seems to have no significance for family or genus. The specific numbers are shown in Table I.

The odd chromosome in the spireme stages differs in its shape in the genus *Aphrophora* from that in the other genera as far as studied. It is a much elongated structure early in its appearance in *Aphrophora*, while it first appears as an oval or round body in the others.

All eight species of the Cercopidæ studied show a typical odd chromosome, which divides only in the second spermatocyte division. In all of the species except *Aphrophora quadrinotata* and *Clastoptera proteus*, in which the material was limited and the equatorial plates consequently not studied in favorable positions, there is one chromosome among the reduced number which is distinctly larger than the others. In no case is the odd chromosome the largest one.

WOODS HOLE,

July 30, 1915.

# THE REACTIONS OF AN ORB-WEAVING SPIDER, *EPEIRA SCLOPETARIA* CLERCK, TO RHYTHMIC VIBRATIONS OF ITS WEB.<sup>1</sup>

WILLIAM MORTON BARROWS.

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## I. INTRODUCTION.

The work reported in this paper was suggested by a chance observation<sup>2</sup> made in the summer of 1911. A fly was held close to one of the spiders without eliciting any response; when the fly's vibrating wing was allowed to touch a strand of the web, however, the response was instantaneous and positive. The spider ran to the fly and seized it. A vibrating rubber band held against a strand of the web caused a very similar response. During the summer of 1913 these spiders were studied more carefully in an attempt to determine: first whether the stimulus was vibratory in nature or must be considered to be due to some other force and second whether the response could be identified as a "tropism" or taxis.

## II. MATERIALS AND METHODS.

At the Lake Laboratory maintained by the Ohio State University at Cedar Point, Ohio, the large orb weaving spider, *Epeira sclopetaria*, is very abundant, building its webs on the front porch and in the angles of the building and roof. The habit

<sup>1</sup> Contribution No. 42 from the Department of Zoology and Entomology, Ohio State University.

<sup>2</sup> The note of Boys (80) was not known to the writer until the larger part of these experiments had been carried out.



of the female of this species of remaining at the center of her web for long periods of time makes it a very convenient form to study in its normal surroundings.

This species builds its web in dead branches or in the angles of buildings where there is an abundance of small or medium sized insects. The web usually consists of 17, 18 or 19 relatively inelastic strands which radiate from a center like the spokes of a wheel. These radiating strands are attached at their outer ends to twigs or boards or to guys or stays which anchor several radii to the support. Surrounding the center of the web is an irregular network known as the hub and notched zone which serves as a resting place for the inhabitant of the web. For a short space outside the hub the radii are bare (the free zone) but beyond this is found the viscid spiral consisting of finer strands which are extremely elastic and are beaded with microscopic sticky drops which serve to hold and entangle the insect prey. It is probably the extreme elasticity of these spiral strands which allows them to detain a strong insect without being snapped, thus giving the spider time to reach the detained insect and complete its ensnarement by the addition of fresh silk from the spinnerets. The normal resting position of the female spider is with the head directed downward and the legs spread outward on the notched zone as is shown in Fig. 1. The method used to obtain this photograph and the others following is that used by Comstock ('12, p. 181). A female spider was placed on a dead branch held in the neck of a bottle which was set in a tray of water. During the first or second night the spider usually built a perfect web. The branch was then moved to the photographing table with as little disturbance as possible and placed in front of a soap box painted a dull black on the inside. Arranged in this way before the camera it was possible to take pictures showing the spider, web, and vibrator straw, and the various positions taken by the spider in the act of responding to the vibrator.

The size of the web varies from two inches in diameter, or even smaller when built by very young spiders, to eighteen inches or more when built by mature females. The male builds a web very much like that made by the female but as he has a roving disposition one is never sure that the same individual can be

located twenty-four hours later while the females often live for weeks in the same place, repairing the web every evening but not altering it materially.

In crawling across the web the spider always follows a radiating strand or at the edge of the web, one of the guy strands, and places its feet on the radii or on the junctions of the radii and spiral threads where the latter hold no sticky materials. The front feet are usually placed on the same radiating strand but the second and third pairs may be spread out on the two adjoining strands. It is possible for the spider to crawl rather swiftly along a single strand for a considerable distance, all eight feet using the same thread. In crossing the web the spider usually leaves behind a dragline which may remain across the web, adhering to it after the spider has returned to the center. Some individuals on the other hand when they reach the edge of the web swing free, held only by the drag line up which they climb in returning to the center. Occasionally one finds both methods employed by the same individual. Most spiders are not skilful enough to cross the web several times without tearing out or snagging several of the segments of the spiral thread. When the web is violently disturbed the spider usually retreats to a niche or corner (the retreat) and remains there motionless unless again disturbed. Some individuals remain in the retreat instead of at the center of the web. When this is done one forefoot is placed on the trapeline leading to the hub and any activity of the web such as that produced by an entangled insect sends the spider like a flash down to the web. In this connection another fact may be noted; a spider outside the center of the orb always returns to the center, takes the normal position and then orients before it finds an entangled insect. This might be explained as due to the difficulty of crossing the web by any other path than by the radii. However, the inability to orient accurately in any other position than the center gives a clue to a more probable explanation. Individuals of the species *Epiara sclopetaria* will eat nearly any insects which happen to become entangled in the web. The food of those studied inside the screened porch consisted almost entirely of rather large flies of the genera *Musca*, *Sarcophaga*, and *Lucilia*. It is in the snaring of these flies that

this *Epeira* seems to be, especially expert. When a fly strikes a web it often goes through, breaking out one or two spiral segments. If, however, it does not break through it hangs for a second, buzzing, then breaks one or two of the sticky strands and flies away. A fly seldom entangles itself to such an extent that it cannot get free inside of five seconds. A successful spider then must reach the fly in less than two or three seconds after it strikes the web. The actual capture of the fly is accomplished usually either by biting the fly and stunning it or by winding it with web. The entangled fly may be left where it struck or may be torn from the web, and carried attached to one of the spider's hind feet to the center of the web where it is thoroughly chewed and its liquid parts swallowed.

The apparatus used to produce rhythmic vibrations consisted of three tuning forks and an electric vibrator. One fork had a vibration rate of 100 double vibrations per second, another a rate of about 487 and the third was an adjustable fork with a large range of vibration rates but with very limited amplitude. The electric vibrator was a modified electric door bell in which the clapper was replaced by a long grass straw. The number of vibrations produced by this instrument could be varied to some extent by changing the tension of a spring and a regulator screw, while the amplitude of the vibration varied with the length of straw used. The vibration rate of the vibrator was obtained by comparing a tracing made by it on a sheet of blackened paper on a revolving drum with a simultaneous record made by the tuning fork giving 100 double vibrations per second. The electric vibrator was found to be more effective than a fork because it gave vibrations of equal intensity, *i. e.*, it did not run down. It had also another advantage in that it could be controlled by a switch held in the hand and could be operated at a distance from the operator. A stop watch was used to measure the time elapsing between the beginning of the stimulus and the arrival of the spider at the place where the straw touched the web.

### III. EXPERIMENTS.

#### 1. *Experiments Using Rhythmic Vibrations.*

When the vibrator straw is placed against one of the spiral strands or against one of the radii and caused to vibrate the spider

orients instantly and advances along the nearest radius to the straw, seizes the straw with its mandibles and may spread web on the straw with the hind pair of feet (Fig. 3). This reaction is carried out in essentially this manner no matter where the straw may strike the web.

The orientation is so rapidly executed and is followed so closely by the forward locomotion that it is difficult to separate the two parts of the response. If, however, the vibrator is set in motion for a fraction of a second only the orienting is accomplished but the forward locomotion toward the vibrator does not follow. A second vibration while the spider is oriented calls forth the forward response and an attack on the vibrator (Fig. 3). The photograph reproduced in Fig. 2 shows such an orientation. If the first vibratory stimulus is not too long or is not followed by a second stimulus the spider usually returns to the resting position at the end of a few seconds. Some individuals, however, follow the orienting response by an interesting series of activities. The fore feet are placed on neighboring radii, drawn toward the animal's body and released suddenly. This release sets the web vibrating parallel to the spider's longitudinal axis. The spider then turns one space to the right or left and repeats the process until she has oriented through a complete circle and set every pair of radii in motion. The use of this activity is seen if there happens to be a captured fly or a piece of dirt in the web. When the two radii which pass on either side of the object are set vibrating the object is also set in motion but its motion is not of the same rate as that of the rest of the web and it sets up an echo or return vibration. To this the spider responds. A dead fly may be rediscovered in this way or a piece of dirt may be located and removed.

Responses to different frequencies show considerable variations and it is not possible to predict that a certain individual will respond in a definite way to a given stimulus. This variation in response ranges from instantaneous orientation and forward locomotion to a slow orientation and slow approach toward the vibrating point or it may happen that no sign will be given that the stimulus has been perceived. Roughly speaking a large spider responds most quickly to a vibration of considerable am-

plitude with a vibration rate of 24 to 300 per second. It was impossible with the materials at hand to construct a vibrator giving a high rate and having also a considerable amplitude, so recourse to steel wires and small forks was necessary. The large spiders did not respond well to wires and forks with high vibration rate and small amplitude but they did respond instantly to the vibrating wings of *Chrysops* (127 per sec.), *Microbembex* (208 per sec.), *Musca* (284 per sec.), where the amplitude ranged from 4 mm. to 10 mm. Small spiders responded quickly to vibrations ranging from 100 per sec. to 487 per sec. and even higher although the amplitude was very small. This difference in responsiveness between the young and old spiders is probably correlated with differences in size and rate of wing vibration of the insects which are ensnared and used as food by young and old. In general small insects have high wing vibration rates while the larger insects have lower rates of wing vibration (Packard, '03, p. 150). The smaller spiders eat small insects and the large spiders eat larger insects. The following species of insects were caught and eaten by *E. sclopetaria*: *Chrysops vitatus* (127 vibr. per sec.); *Calliphora vomitaria* (130 vibr. per sec.); *Microbembex monodonta* (208 vibr. per sec.); *Musca domestica* (284 vibr. per sec.). Many small midges (*Chironomus* and others) were eaten by the young spiders and occasionally by the adults. The vibration rate of these small midges is probably very high, judged by the high pitched note which they give out, but it was impossible at the time to determine its rate.

## 2. Experiments Using a Y-shaped Vibrator.

In order to determine whether the spider reacted to a single vibrating strand or to the center of a vibrating area of the web, a Y-shaped vibrator made up of insulated magnet wire was adjusted to the vibrator and arranged in such a manner that its ends touched the web at two places, 2 or 3 cm. apart. When the vibrator so adjusted was operated the spider responded readily, going to a point on the edge of the web midway between the two vibrating points and then after some slight hesitation going toward one or the other of the vibrator wires (see Fig. 4). If, however, these points of wire were more than 3 cm. apart the spider at the



center of the web usually hesitated, turning first toward one, then toward the other, finally orienting to one and attacking this by itself.

### 3. *Response in the Dark.*

In order to test the ability to respond in the dark the vibrator was set up late in the afternoon, the straw touching one of the radial strands of a web which was built in the frame of a window. The window was shaded on the outside by a heavy thicket. At 9:30 P.M. the room was so dark that a person standing inside could discern the outline of the window with the utmost difficulty. A flash of light from a pocket electric lamp showed that the female occupying the web was at the center of her web. The vibrator switch was closed and at the end of about four seconds the electric flash light showed the spider biting the vibrator straw in the same manner as that shown in Fig. 3. This experiment indicates that unless these spiders use rays of light which our eyes do not perceive, sight plays no essential part in the orientation to and the ensnaring of the prey.

### 4. *The Distribution of Vibrations through the Web.*

The distribution of vibrations as they travel across the web is of some theoretical interest. The following method for recording these vibrations was used with considerable success. A spider in its web was placed before the camera and made to respond to the vibrator repeatedly until it would respond no more. A photograph (Fig. 5) of 15 seconds' exposure was then made while the vibrator was in motion. The web was somewhat torn by the spider before it ceased to respond, but the photograph reveals by the thickening of the lines the distribution and amplitude of the vibrations in all parts of the web. The amplitude of the vibrations decreases rapidly from the periphery toward the center. The radial strand connected with the vibrator shows the greatest lateral displacement while the strands on either side of this show less and less disturbance as the distance away from the vibrator increases. A slight thickening of the spiral strands in a direction at right angles to the direction of the primary vibration can be noted on the segments directly across the center from the vibrator. The center of the web seems to be the part

least affected. If there is any motion here it is probably at right angles to the original vibration, that is, it is probably parallel to the spiders' long axis after orientation.

##### 5. *Mutilation Experiments.*

The foregoing experiments coupled with careful observations on the spinning behavior of the orb-weaver lead to the conviction that the organs used in detecting the movements of the web are probably tactile, at least there are no other organs described which would seem to serve the purpose as well. There can be little doubt that sense hairs are very abundant on the legs, particularly on the tarsi of these spiders. These hairs have been described by Dahl (83), Wagner (88), McCook (90), and recently by McIndoo (11). The functions of these hairs have been interpreted in various ways, but little or no experimental work has been accomplished other than attempts to show that some spiders hear. Responses to sounds seem to have been observed only in those forms which build webs. It seems likely that responses in the web building forms are due to the vibrations of the air being picked up by the strands of the web (McIndoo, '11, p. 412). It was thought desirable to determine if possible the location of the sense-organs used in detecting vibrations. By careful manipulation with a pair of fine dissecting scissors it was possible to snip off one or more of a spider's legs without causing the spider to leave the web. It is necessary to use great care not to shake the web because an irregular shaking gives rise to the negative response, the spider running away to the retreat. The contrast between this insensibility to the amputation of legs and extreme sensitiveness to irregular vibrations of the web emphasizes the fact that these spiders receive most if not all of their mechanical stimuli through the web. These operations caused the spider to lose considerable blood but two or three hours usually sufficed to heal the wound. The stumps of the legs were always held up so that they did not touch the web.

*Experiment 1.*—After testing a spider to be assured that its responses were normal the two forelegs were cut off as near the middle of the metatarsus as possible. This spider immediately put the stumps of the forelegs into its mouth. The next morning



this spider was in its web. During the night the web had been repaired and a new spiral thread put on.

In recording the test made on this spider and those following, IX o'clock, XII o'clock, etc., refers to the position at the edge of the web which corresponds to the same hour on the clock face. Thus VI o'clock is used to designate the edge of the web which the spider normally faces when at rest, *i. e.*, directly downward.

*Experiment 1.*—Spider with both forefeet cut off. Fork giving 100 vibrations per second touching web in  
III o'clock position spider reached fork 8 inches from center in 3 seconds.

IX o'clock position reached fork (8 in.) in  $2\frac{1}{2}$  sec.

VII o'clock position reached fork (8 in.) in  $2\frac{1}{2}$  sec.

*Experiment 2.*—Spider with third legs cut between femur and patella.

IX o'clock position reacted 8 in. in  $2\frac{1}{4}$  sec.

III o'clock position reacted 8 in. in  $2\frac{1}{4}$  sec.

XII o'clock position reacted 8 in. in  $7\frac{1}{4}$  sec.

This individual showed some difficulty in climbing, but oriented accurately.

*Experiment 3.*—Spider with second legs cut off at patella.

III o'clock position reacted 7 in. in  $1\frac{1}{2}$  sec.

XII o'clock position reacted 7 in. in  $1\frac{1}{2}$  sec.

X o'clock position reacted 7 in. in  $1\frac{1}{2}$  sec.

*Experiment 4.*—Spider with fourth legs cut off at patella. Reactions entirely normal as given above.

Another set of experiments which need not be detailed were carried out. In these the right first leg and left fourth leg were cut off and other similar combinations were made. In all cases orientations and the locomotion following were entirely normal except for the slight difficulties in locomotion which might be expected. These experiments indicate that the sense organs used in reacting to the vibratory stimuli are not restricted to any one pair of legs below the metatarsus. There are two possible distributions of sense hairs which would seem to make possible the reactions detailed above; the sense organs may be confined to the feet, where they come in contact with the web or they may be located on the legs or body in such a manner that they pick up

the vibration of the whole leg or whole body. Hinged sensitive hairs uniformly scattered over the body might answer this purpose. It seems most likely, everything considered, that the particular sense organs used are on the tarsi of each leg and come in contact with the web. It is difficult to conceive that an animal whose feet are not extremely sensitive could travel on or manipulate the delicate strands of these orb-webs.

#### IV. DISCUSSION AND SUMMARY.

It is maintained in this paper as in a previous one (Barrows, '07) that an animal exhibits a "tropism" or better a taxis, "when under the influence of [chemical] stimuli acting unilaterally they move toward or away from the source of the stimulus" (Verworn, '99, p. 249). It has been shown above that *Epeira sclopetaria* orients in its web and moves toward the source of a vibratory mechanical stimulus when this is of an appropriate rate and amplitude. Thus this method of response to a vibratory stimulus identifies the reaction as a positive taxis. The term tonotaxis would naturally be used in this connection, but since tonotaxis has been used in another way it seems advisable that the terms positive vibrotaxis should be applied if a short descriptive term is desired.

The foregoing may be summarized as follows:

1. *Epeira sclopetaria*, an orb-weaving spider, starting from the center of its web is able to orient, charge and seize flies which strike and are detained in the web. This process is carried out with extreme rapidity.

2. With the aid of a mechanical vibrator it is possible to show that the stimulus is vibratory, the spider orienting to and attacking the vibrator even in the dark.

3. The response can be analyzed into, (*a*) the orientation, (*b*) the forward response, and (*c*) the attack on the vibrating object. The response is in essence a positive vibrotaxis.

4. The vibrations are transmitted through the web in all directions from the vibrating point but the intensity (amplitude) decreases toward the center of the web and on either side. The lines of equal intensity of the vibration form roughly a series of circles the centers of which are at the vibrating point.

5. The sense organs used in detecting the stimulus are probably sense hairs on the tarsi.

6. This orb-weaving spider provides itself with a temporary extension of its tactile sense organs which makes its tactile sense in reality a distance receptor, much like an auditory or an olfactory organ.

#### BIBLIOGRAPHY.

**Barrows, W. M.**

'07 The Reactions of the Pomace Fly; *Drosophila ampelophila* Loew, to Odorous Substances. *Jour. Exp. Zool.*, Vol. IV., No. 4, p. 515-537.

**Boys, C. V.**

'80 The influence of a Tuning Fork on the Garden Spider. *Nature*, Vol. 23 p. 149.

**Comstock, J. H.**

'12 The Spider Book. Garden City, N. Y.

**Dahl, T.**

'83 Ueber die Hörharre bei den Arachniden. *Zool. Anz.*, VI.

**Davenport, C. B.**

'08 Experimental Morphology, New York.

**McCook, H. C.**

'90 American Spiders and their Spinning Work. Vol. II. Philadelphia.

**McIndoo, N. E.**

'11 The Lyriform Organs and Tactile Hairs of Araneads. *Proc. Acad. Nat. Sci. Phil.*, May, 1911.

**Packard, A. S.**

'03 A Text-book of Entomology, New York.

**Peckham, G. W. and E. G.**

'87 Some Observations on the Mental Powers of Spiders. *Jr. Morph.*, I., pp 383-419.

**Verworn, M.**

'99 General Physiology. Trans. by F. S. Lee. London, 8vo. xvi+615 pp.



## EXPLANATION OF PLATE I.

FIG. 1. Showing a female *Epeira scolopetaria* in the normal resting position in the web. The arrow indicates the place where the vibrator straw touches a radial strand of the web.

FIG. 2. The same individual, shown in Fig. 1, orienting to the vibrator which had been in motion for a fraction of a second just before the photograph was taken.

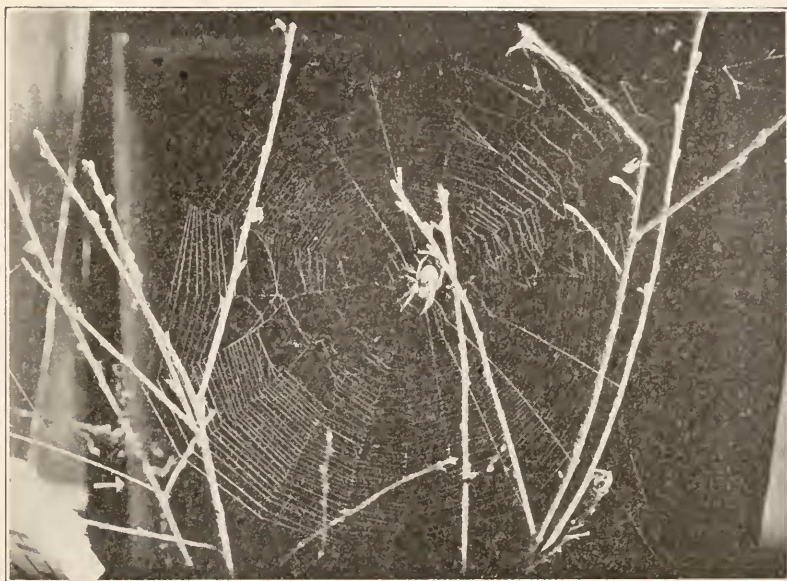


FIG. 1.



FIG. 2.

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## EXPLANATION OF PLATE II.

FIG. 3. A spider attacking the vibrator straw while it is in motion.

FIG. 4. A spider in the act of responding to the Y-shaped vibrator. One prong of the vibrator appears in front, the other behind the spider.

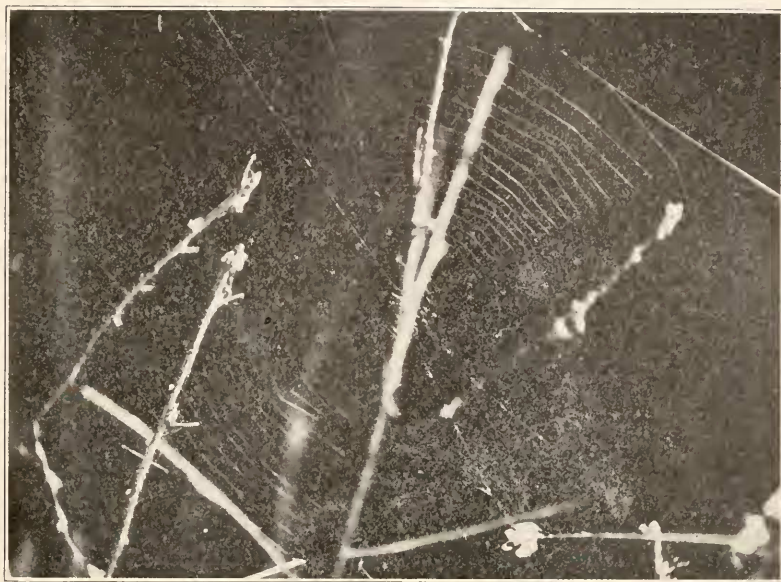


FIG. 3.



FIG. 4.





## EXPLANATION OF PLATE III.

FIG. 5. A photograph showing the spider in the normal resting position in the web, while the vibrator is in motion. The arrow indicates the place where the vibrator straw touches a radial strand. The doubling or blurring of the lines of the web shows the distribution of the vibrations.



FIG. 5.

W. M. BARROWS.





# BIOLOGICAL BULLETIN

## OBSERVATIONS ON THE DEVELOPMENT OF COPIDOSOMA GELECHIAE.

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*From the Marine Biological Laboratory, and the Zoological Laboratory of the University of Texas (Contribution No. 127).*

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### I. INTRODUCTION.

The discovery of polyembryonic development among certain of the hymenopterous parasites has opened up an extremely interesting field for investigation. Like most other important biological discoveries, this one was foreshadowed by the observations of several different naturalists. In a paper of this nature it is not necessary to give an extended account of the history of this discovery. We shall therefore be content with a brief

statement on this point, limiting the account almost entirely to the species with which the paper deals.

The general features of polyembryony in insects have been given in the well-known papers of Marchal ('98, '04) and Silverstri ('06, '08), but there are many points concerning the details of this process which have not as yet been worked out. It was with the view of studying certain of these details that led the writer three years ago to seek an American species upon which such studies could be made. Dr. L. O. Howard<sup>1</sup> suggested that *Copidosoma gelechiæ*, which parasitizes the larvæ of the *Solidago* gall moth, *Gnorimoschema gallæsolidaginis*, would be a good form upon which to work, as it seemed to be an undoubted case of polyembryony.

The *Gnorimoschema* moth makes the ellipsoidal galls on the stems of several species of goldenrod. Baron Osten Sacken ('63) seems to have been the first to have published a description of the inflated carcass of the *Gnorimoschema* larva, caused by the chalcis parasite; but apparently he was not acquainted with the maker of the gall. In 1869 in connection with his account on the life history of this moth, Riley states that the caterpillar serves as a host for no less than six different species of hymenopterous parasites. One of these, which is shown in his Fig. 6, Plate 2, is described as a "little fly of a dark metallic green color, with reddish legs." This is clearly *Copidosoma*. Riley states that the larvæ of this species infests the caterpillar in great numbers, more than 150 having been obtained from a single host. He supposed that the "mother fly" gnawed a passage through the gall and desposited her batch of eggs in the inmate. He pointed out that the larval parasites cause the caterpillar to swell to three or four times its natural size, and after having absorbed all the juices of the victim, form very small brownish cocoons, which are so packed together that they give to the worm the puffed-up appearance which is typical of the mummified carcasses of lepidopterous larvæ that have been parasitized by a polyembryonic species. It was this inflated condition of the larval host that led Riley to call the parasite the "Inflating Chalcis

<sup>1</sup> For this as well as for other suggestions received throughout the progress of the work, the writer is greatly indebted to Dr. Howard.

Fly." Howard ('85) later named this species *Copidosoma gelechiæ*.

Upon examining the various goldenrods about Woods Hole, Mass., for galls of *Gnorimoschema*, it was found that *Solidago sempervirens* furnished the best opportunity for obtaining material. However, the common gall maker of this solidago proved not to be *Gnorimoschema gallæsolidaginis* Riley, but a closely related species, *G. salinaris* Busck. The parasites infesting these two moths are varieties of the same species, *Copidosoma gelechiæ*.

The selection of this species has not proved altogether satisfactory, because the gall-making habit of the host complicates the life history and renders the collecting of material for early stages of the parasite somewhat more difficult than from a host which feeds openly. Furthermore, the moth, and likewise the parasite, has but one generation a year. In addition to these objections, there is the further one that the egg of *Copidosoma* gives rise to a relatively large number of individuals (about 191 on the average). In attempting to obtain material for the studies which the writer has in mind, it seems best to seek to find a host which is an open or semi-open feeder, which has two or more generations a year, and which harbors a parasitic egg giving rise to but few individuals. During the past summer at least two species have been found which in the main seem to fulfill these conditions. It therefore seems best to publish the main facts concerning the development of *Copidosoma* before giving it up for more favorable material.

There is one feature in the development of *Copidosoma* which makes further study desirable. We refer to the abortive embryos (presently to be described), which at first were thought to be comparable to the so-called asexual larvæ of *Litomastix truncatellus*. It will be recalled that Silvestri ('06) described in this species the development of both sexual and asexual larvæ from a single egg. In one instance he secured from a caterpillar of *Plusia gamma* 1,700 sexual and 220 asexual larvæ of *Litomastix*. He believes that the asexual larvæ play the rôle of rasps for the normal larvæ, tearing the tissues of the host so that the sexual larvæ may the more easily secure the necessary food. It may

be stated here that the abortive larvæ of *Copidosoma* are in no way comparable to the asexual larvæ of *Litomastix* as described by Silvestri.

## II. NOTE ON THE LIFE HISTORY OF GNORIMOSCHEMA.

In order to collect polyembryonic material it is essential to know something about the life history of the host; especially is this true in cases like *Gnorimoschema* in which the larval host is a gall maker. Considerable attention has therefore been given to a study of the life history of *G. salinaris*.

The general habits of the *Solidago* gall moths were first made known by Riley's ('69) studies on *G. gallæsolidaginis*. According to Riley this species winters over in the imago stage and may be seen flying in the month of May. When the young plants (*Solidago nemoralis*) are about six inches high the female moth lays her egg either in the terminal bud or at the side of the stalk immediately below the bud. The young caterpillar upon hatching burrows into the stalk and starts the development of the gall. By the first of June the gall has just begun to form and contains a larva about one-third grown. The larva and its ellipsoidal gall reach their full size by the middle of July. The caterpillar which now measures over half an inch in length prepares for the change into the chrysalis state by first eating a round passage-way through the wall well toward the upper end of the gall. The orifice is then closed by a secretion of liquid silk, which hardens to form a silken plug. After closing the orifice, the caterpillar lines the passage-way and the walls with a delicate silk, and then transforms into a shiny, mahogany-brown pupa, about a half inch long. The moths begin to emerge about the middle of August and continue to appear until the beginning of October.

Many phases of the life history of *G. salinaris* are similar to those of *G. gallæsolidaginis*, but there are some important differences. The earliest date at which galls of the marsh goldenrod have been secured was June 12, 1914, and at that time many of the galls were well started. Between June 12 and 15, 63 galls of various sizes were collected and examined. They varied in size from 8 to 12 mm. in length and from 4 to 17 mm. in transverse section. In shape the galls also vary greatly. Some are distinctly

pear-shaped, while others are fusiform, with various gradations between these two general types. The galls occur at different heights on the stem, but the vast majority of them are located at or near the base of the stalk (Fig. 1). Their position is undoubtedly determined by the location of the point at which the larva penetrates the young shoot. If this point is located toward the base of the young stalk, the gall will naturally appear near the base of the fully grown plant; but if it is located in or near the terminal bud, the gall will appear some little distance up on the stem. Occasionally two galls are found on the same plant (Fig. 8). A few cases have been observed in which the gall was located at the tip of the terminal bud, producing a stunted plant without a central, flower-bearing stalk. With these few exceptions, the gall of *G. salinaris* does not seem materially to affect the growth and vigor of the plant. It is true that many galls are found on plants that are apparently stunted but such dwarfing is to be attributed to the adverse conditions under which the plant sometimes grows. In regions that are very much exposed to the wind, like the banks along the coast, many of the goldenrods are small and clearly dwarfed; but this condition applies as well to the plants that are free from galls as to those that are infected.

The habits of gall making are similar in the three common species of *Gnorimoschema*, although the following differences may be pointed out. *G. gallæasteriella* produces a triangular gall at the top of the dwarfed or stunted stems of *Solidago cæsia*, *S. axillaris*, *S. latifolis*, and *Aster divaricatus*.<sup>1</sup> The form of the gall differs somewhat with the plant. The gall of *G. gallæsolidaginis* may occur toward the top of the stem, but usually it is located just below the middle, especially is this true of the galls on *S. canadensis*. The galls of this moth do not dwarf the plant. The condition of the galls of *G. salinaris* on the marsh goldenrod has already been described. They occur nearer the base of the stem than do those of last species, and like the latter there is little or no tendency to dwarfing the plant.

The larvæ secured from the galls collected between June 12 and 15 varied from 3 to 8 mm. in length. Beginning with the middle of June, the young caterpillars grow rapidly, doubling

<sup>1</sup> Part of these data were kindly furnished the writer by Dr. T. M. Forbes.



their size within a fortnight. By the middle of July they have reached their full growth, and are beginning to show signs of undergoing pupation, which is evidenced by the construction of the passage-way. The passage-way and its orifice differ in two respects from those of *G. gallæsolidaginis* as described by Riley ('69). The silk lining does not extend much beyond the lower limits of the passage-way, and hence does not cover the inner surface of the wall. The second difference is seen in the character of the orifice and its silk plug. The caterpillar of *G. salinaris* does not cut the passage-way quite through the wall, but leaves the very thin epidermis of the stem, which is used as a background for the construction of the plug (Fig. 7).

TABLE I.

TABLE SHOWING DATES OF PUPATION AND EMERGENCE OF COPIDOSOMA AND GNORIMOSHEMA.

Pupation (Beginning of)	Copidosoma	{ Aug. 6, 1912. Aug. 5, 1913. July 31, 1914. July 30, 1915. Aug. 6, 1912.
	Gnorimoschema	{ July 23, 1913. July 30, 1914. July 26, 1915. Aug. 25 to Sept. 12, 1912.
Emergence	Copidosoma	{ Sept. 3 to Sept. 13, 1913. Aug. 30 to Sept. 18, 1914. Aug. 24 to Sept. 21, 1915.
	Gnorimoschema	{ Aug. 25 to Sept. 10, 1912. Aug. 25 to Sept. 10, 1913. Aug. 22 to Sept. 11, 1914. Aug. 24 to Sept. 14, 1915.

Pupation occurs during the last week of July and the first week of August (Table I.). The imagines begin to emerge about August 25, and continue to appear until September 10. The moth has been seen flying in the open during this period.

Females kept in captivity often lay eggs. This they do within ten days after emerging, and irrespective of their association with males. As a rule the moths simply drop the eggs on the bottom of the cage, or they may lay them on the leaves and flowers of goldenrods placed in the cage. At first it was thought that *G. salinaris* must differ from *G. gallæsolidaginis* in respect to its egg-laying habits, for Riley states that the latter species although emerging in the fall, hibernates as an imago and lays



its eggs in the following May. It has been discovered, however, that *G. gallæsolidaginis* from the galls of *S. canadensis* in western Ohio likewise drops several eggs soon after emerging from the pupa in September. This raises the question as to whether these fall eggs develop into larvæ, for if so it would be difficult to explain how the young caterpillars could withstand the winter and succeed in the spring in finding a young goldenrod bud or shoot in which to start the new gall.

In reply to an inquiry, Mr. A. Busck of Washington kindly informed the writer that the laying of eggs by *Gnorimoschema* was of no particular significance, as it is not uncommon for certain Lepidoptera to drop their eggs prematurely, especially if kept in captivity. In view of this fact an observation made in the fall of 1913 is of special interest. During the first few days of September of that year a single female, confined in a cage with several males, laid a dozen or more eggs on goldenrod leaves and flowers. On the thirteenth of the month three larvæ hatched from this batch of eggs! There can be no possible doubt as to the correctness of this observation, for the hatching of one of the little caterpillars was actually observed under a hand lens.

It is difficult to explain the development of these larvæ from fall eggs, except on the basis of parthenogenesis. It is true that the female which laid the eggs from which the larvæ developed had been confined with males; but although males and females have been kept together for several weeks during each of the last three seasons, yet mating has never been observed. The supposition that the fall eggs of *G. salinaris* may develop by parthenogenesis receives strong support from a study of sections of eggs laid by a female not associated with males. In Fig. 20 is shown a transverse section of one of her eggs and it can clearly be seen that development is well started. Twelve eggs out of the batch were sectioned, and it was found that eleven had started to develop, although apparently not in a normal manner. It is not improbable that some few eggs may develop normally and eventually produce larvæ. The question of parthenogenesis in the *Solidago* moths is one needing further study.

It might be worth while to add that parthenogenetic development among Lepidoptera is by no means unknown. DeGeer is

given credit for having first discovered long ago that certain butterflies belonging to the genus *Solenobia* lay unfertilized eggs which develop into normal imagines, and later von Siebold not only confirmed this observation, but also discovered that *Psyche helix* reproduced parthenogenetically. It has since been shown by several workers that the silk moth, *Bombyx mori*, may under certain conditions reproduce by parthenogenesis.

### III. PARASITES OF GNORIMOSCHEMA SALINARIS.

Riley reports six hymenopterous parasites for *Gnorimoschema gallæsolidaginis*, and in addition to these he found a beetle larva and another lepidopterous larva which intrude as inquilines within the cavity of the gall made by this species. At least five hymenopterous parasites have been found associated with *G. salinaris*. The most important of these is *Copidosoma gelechiæ*, which is by far the most common parasite attacking the moth. The other four species are *Calliephialtes notanda* Cress, *Epiurus* sp., *Eurytoma* sp. (pupa), and *Pseudacrias sexdentatus* Girault. The first of these four occurs most frequently, while the last has been observed but a few times. However, it is of special interest, inasmuch as it is the only observed case of a second parasite emerging along with *Copidosoma*, although the larvæ of other species have been found associated with the larvæ of *Copidosoma*. On September 3, 1914, six individuals, all females, emerged together with a brood of about one hundred *Copidosomas* from a single carcass. The small pupæ of *Pseudacrias* lying among those of *Copidosoma* were observed through the transparent chitin of the carcass of the host some days prior to their emergence. They were not grouped together but scattered about in different parts of the carcass. Each pupa was inclosed in a chamber very much smaller than, but exactly similar to that containing a *Copidosoma* pupa.

Usually *Pseudacrias* larvæ do not pupate until after the larval host has undergone this process. About a dozen *Gnorimoschema* pupæ have been found containing *Pseudacrias* pupæ, which later emerged. It is not probable that *Pseudacrias* is polyembryonic. First, because both male and female individuals usually emerge from the same pupal host; and second, because the individuals

do not come out at the same time. The single instance of six females ssuing simultaneously with the brood of *Copidosoma* can be explained by assuming that a single female deposited six fertilized eggs in the host at the same time. However, this case is of special interest as it demonstrates the synchronous development in a single host of the broods of two distinct parasites, and thus supports Wheeler's ('10) suggested explanation of Silvestri's so-called asexual larvæ in *Litomastix*.

In addition to the five hymenopterous parasites, there are two insect larvæ associated with the larva of *G. salinaris*. They are undoubtedly inquilines. One of these is a beetle and the other a lepidopterous larva (Fig. 5). Judging from Riley's account, these two species are very similar to if not identical with the corresponding inquilines reported by him for the galls of *G. gallæ-solidaginis*.

#### IV. DEVELOPMENT OF COPIDOSOMA GELECHLÆ.

##### 1. *The Polygerm Stages.*

(a) *Youngest Stages.*—We have not secured the cleavage stages of *Copidosoma*, owing to the fact that they occur earlier in the year than we have been able to reach Woods Hole. Therefore, in describing the developmental processes which have their inception in the cleavage stages, we must rely upon the work of other investigators in this field for our interpretation of the significance of these processes.

The youngest stages secured were found in a small larva of *Gnorimoschema*, taken June 21, 1913. The series of sections of this small caterpillar contains three young polygerms of *Copidosoma*. Evidently the egg from which the caterpillar developed had had three parasitic eggs deposited in it. Two of the polygerms, which lie close together, are situated in the first and second body segments of the larva, just beneath the hypodermis; while the third is found in sections 5 to 14 posterior to these, and is also situated just beneath the hypodermis of the host.

The three polygerms are not of the same size, as is indicated by the following measurements: Of the two specimens lying close together, the larger measures  $150\ \mu$  by  $82\ \mu$  and runs through 15 sections ( $150\ \mu$ ), the smaller measures  $103\ \mu$  by  $71\ \mu$ , and is

found in 12 sections; the single specimen measures  $179\ \mu$  by  $95\ \mu$  and occupies 8 sections only.

In structure the three polygerms are practically identical. Each consists of two distinct zones: (1) An outer relatively thick zone containing a large number of nuclei irregularly placed, and (2) a central region containing the embryonic nuclei (Fig. 19). In the absence of the earlier stages, it is not an easy matter to interpret the conditions seen in these polygerms. In the main they correspond most nearly to the conditions in the egg of *Litomastix* (*Copidosoma*) *truncatellus*, as described by Silvestri ('06). I therefore interpret the outer zone to be the product of the "polar oöplasm" plus the "polar nuclei," while the central region contains the true embryonic nuclei, derived from the fertilized nucleus, or in the case of parthenogenetic development, from the matured egg nucleus.

There is of course one essential difference in the corresponding stages of *Litomastix* and *Copidosoma*. In the polygerm of the former the central region is composed of a solid mass consisting of distinct cells, while in the latter this region is on the point of being broken into multi-nucleated masses, which form the primordia of the embryos (cf. Fig. 19 A with Silvestri's Fig. 33, Pl. III.). It may be that the embryonic nuclei are delimited by cell walls in *Copidosoma*, although one can not make them out with certainty, even under the highest powers of the microscope. Judging from the work of other investigators, one would expect to find the embryonic nuclei surrounded by cell walls. In *Ageniaspis*, Marchal ('04) first reported that the early embryonal masses were pluri-nuclear in character, but Silvestri ('08) and Martin ('14) have later demonstrated that the nuclei are surrounded by cell walls. In *Copidosoma* the embryonic nuclei are often so closely packed together that the demonstration of cell walls would be extremely difficult.

The three polygerms mentioned above are of particular interest, in that they show very clearly the manner in which the central mass with its nuclei breaks up to form the primordia of the multiple embryos. The central region itself consists of two distinct substances. (1) A granular protoplasm in which the embryonic nuclei lie, and (2) a more fluid-like material which

becomes greatly shrunken during the process of fixation, and which in sections appears as a precipitated substance (Fig. 19 *A*, *P.M.*). As to the origin of these different substances we know nothing, but their subsequent history is clear. For the sake of clearness in description we shall use the following terms: (1) *Nucleated Membrane* for the outer zone; (2) *Granular Layer* for the protoplasm containing the embryonic nuclei; and (3) *Precipitated Material* for the shrunken fluid-like substance.

(b) *The Nucleated Membrane*.—In these young polygerms the outer zone stains more deeply than the central mass. The “polar nuclei” have no definite arrangement, but are irregularly scattered throughout the protoplasm. The entire zone therefore is in every sense of the word a syncytium. As the polygerm grows in size the nuclei become arranged into a single layer, and the protoplasm thins out, thus forming a true nucleated membrane about the central or embryonic portion of the egg (Fig. 21, *N.M.*). In the later history of the polygerm some of the nuclei are clearly surrounded by cell walls, that is, there is a tendency for the membrane to become cellular.

At first the young polygerms are naked, that is there are no elements from the host tissue laid down on the outer surface of the nucleated membrane. Later a few mesenchyme cells are found on the surface of the membrane, and still later these cells give rise to the adipose tissue (Fig. 22, *A.T.*), which may completely surround the polygerm.

(c) *Precipitated Material*.—This material occupies the central portion of the polygerm. Apparently it is formed through the action of the fixing reagent upon the fluid-like protoplasm. In sections it is very much shrunken, thus leaving an irregular clear space (Fig. 21, *C*). As we shall see later, it persists throughout the entire polygerm phase of development.

(d) *The Granular Protoplasm and the Embryonic Nuclei*.—In Fig. 19 the condition of the embryonic nuclei and their surrounding granular protoplasm is especially clear. Most of the nuclei are indifferently scattered in the protoplasm, but some of them are collecting into groups. The number of nuclei in each group is variable; some groups contain only two or three nuclei, while others may have as many as ten or twelve. The granular pro-



toplasm surrounding a group of nuclei soon rounds off and the primordial embryo with its surrounding layer lies free within the more fluid contents of the central region of the egg (Fig. 19 A). The more usual condition is for the spherical mass to remain attached at one side to the peripheral layer of the granular protoplasm (Fig. 19 B, P.E.). Eventually all of the embryonic nuclei thus become included in these spherical masses of protoplasm, and thus become isolated as primordia of the embryos.

The condition at the close of the formation of the primordia is shown in Fig. 21. This specimen was found in a series of sections of a 3 mm. caterpillar, taken June 15, 1914. In the median section it measures  $113\ \mu$  by  $203\ \mu$ , and runs through 40 sections ( $280\ \mu$ ). It lies in the middle portion of the body cavity, to one side of the intestine, which on account of the size of the polygerm is pushed out of place. As compared with the preceding polygerms this one is very much larger and shows a number of important changes. The nucleated membrane has become much thinner and its nuclei are arranged more or less into a single layer. The adipose tissue is being laid down on the outer surface of the membrane. The most important change, however, has occurred in the embryonic masses themselves. The protoplasm which surrounds a group of nuclei is differentiated into two distinct regions. The central part, crowded with nuclei, stains somewhat lighter than the peripheral zone, which forms a relatively dense layer about the central core (Fig. 21, P.E.). There are still a few nuclei which have not as yet been surrounded by the dense layer, but this stage marks approximately the end of the division of the germ into separate embryos.

(e) *Growth of the Polygerm and the Formation of the Primary Divisions or Masses.*—Upon the completion of the primitive embryos, the polygerm grows very rapidly. It first extends in the direction of its long axis, soon transforming into an elongated cylindrical structure. One specimen showing this condition measures in section  $148\ \mu$  by  $430\ \mu$ . It never becomes an elongated tube as does the polygerm of *Agéniaspis*. During this growth the adipose tissue is laid down in the form of a thick layer about the polygerm. One of the easiest ways in which to find a polygerm of this and later stages is to examine the large

fat bodies lying in the middle region of the body cavity of the larval host. If the caterpillar is parasitized one of these bodies is almost certain to contain the polygerm.

After the elongated condition is attained, the further growth of the polygerm may take place in any direction. In some cases the extension is mainly in one plane, thus transforming the polygerm into a flat, plate-like structure (Fig. 13). In other cases it forms a thick irregular mass (Fig. 11), and when viewed as a whole mount shows many elevations on its surface, due to the breaking up of the entire polygerm into single masses, each of which contains an embryo.

During the rapid expansion of the polygerm a very important change takes place in its structure, whereby each embryo become enclosed in a double involucre. The first step in this process begins just prior to that period of development in which the polygerm attains its elongated, cylindrical shape. It consists in the formation of constrictions in the nucleated membrane which break up the single polygerm into a series of primary divisions or masses (Fig. 15). In the specimen shown in this figure there are about twelve of these masses. Each primary mass has the same general structure as the original single polygerm. It is surrounded by a portion of the nucleated membrane, contains precipitated material, and has a variable number of embryos, from five to fifteen or more.

In Fig. 22 one end of a longitudinal section of a polygerm is shown with the completed primary masses. Three of these masses are seen in the figure, together with a portion of a fourth. Attention should be called to the fact that the adipose tissue, although in contact with the polygerm, is still a distinct structure. In the process of forming the primary masses not all of the elements of the nucleated membrane are taken into these structures. Some of them are left behind and later lie in the inter-embryonal spaces or interstices. In Fig. 22 a number of these elements (cells and nuclei) are shown at the point marked "N," lying between the primary masses and the adipose layer.

In another portion of the same polygerm a single primary mass is being constricted off laterally. It appears as a bud extending from the main body of the polygerm. It is such cases



as this which give rise to the condition frequently seen in whole mounts, in which the surface of the polygerm displays many protuberances.

(f) *Formation of the Secondary Masses.*—The primary masses soon become broken up into secondary masses. This is also brought about by constrictions of the nucleated membrane (Fig. 23). These secondary masses may contain more than one embryo, in which case they immediately form constrictions which result in producing still smaller masses, each of which contains a single embryo.

In the constrictions which lead to the cutting off of a single embryo with its involucre, some of the precipitated material is enclosed between that portion of the granular layer which is in contact with the embryo and that part lying adjacent to the inner surface of the nucleated membrane. These two parts of the granular layer then fuse, forming a single involucre in which are the spaces containing the precipitated material (Fig. 24). The embryo is thus surrounded by two involucre, a granular layer, and a nucleated membrane (Fig. 26). In some cases the precipitated material may be so abundant as to form a solid zone between the inner and outer parts of the granular layer; in others it is small in amount and gives the appearance of much flattened nuclei lying within this layer (Fig. 26, *P.M.*).

(g) *The Inter-embryonal Substance.*—At the close of the formation of the single embryonic masses and their involucre the inter-embryonal interstices are already filled with a substance derived from several different sources. It consists of a plasma-like matrix in which are embedded cells and nuclei. We have already noted that during the formation of the primary and secondary masses some of the elements from the nucleated membrane are not included in the outer involucre, but are left in the inter-embryonal spaces. During the early history of the inter-embryonal substance, it consists mainly of product from this membrane. Later cells from two other sources enter into its formation. First, leucocytes from the host are found embedded in the matrix. They are especially abundant in those regions of the polygerm exposed directly to the body cavity, that is near a surface barren of adipose tissue. Second, fat cells

from the adipose layer invade the inter-embryonal spaces. The fat cells are the last elements to enter the inter-embryonal substance. In Fig. 13 a wedge-shaped mass of fat tissue is seen lying between the embryos in the middle region of the polygerm, on the upper side. Perhaps it would be more correct to say that the embryos bud out into the adipose tissue. Thus in Fig. 24 a single primary mass has been budded off into the adipose tissue.

The final condition of the polygerm at the end of the formation of the inter-embryonal substance is shown in Fig. 16. The adipose tissue has invaded the inter-embryonal substance from all sides of the polygerm and has become an organic part of this substance. The fat body and the included polygerm become an extremely complex structure, which may be called the *polygermal mass*.

## 2. *Dissociation of the Polygermal Mass.*

The setting free of the larval parasites into the body cavity of the host is brought about through the dissociation or disintegration of the inter-embryonal substance. The fat brought into close contact with the embryos by the invasion of the adipose tissue is digested and absorbed by them. It is therefore the first component of the inter-embryonal substance to disappear. That the fat is digested and consumed by the embryos is evidenced by the fact that the numerous other fat bodies remain intact during this period. The disappearance of the fat leaves the embryos loosely held together by the plasmalike matrix, which in turn soon disintegrates, freeing the larvæ.

The first larvæ to be set free are those situated at the periphery of the polygermal mass. Such larvæ are usually the largest present in the mass. As the inter-embryonal substance slowly disintegrates the remainder of the larvæ are gradually set free (Fig. 17). The earliest date at which free larvæ have been found was July 19; the latest, July 31. In the vast majority of cases the mass dissociates during the last week of July.

The larvæ retain the involucre for some time after being set free (Fig. 18). Once free in the body cavity they proceed to devour the contents of the host, first consuming the fat tissue, and finally the various organs. The last internal organ to disappear is the intestine.

### 3. *Pupation, and the Emergence of the Imagines.*

Pupation in *Copidosoma* occurs during the first ten days of August. The pupa stage lasts twenty-eight days. As stated above, the larvæ destroy all of the internal organs of the host, and consume such portions as are dissolved by the action of their salivary secretions. The undissolved portion consists largely of the chitinous parts of the tracheæ. The larvæ also destroy all of the body wall except the superficial layer of chitin. During the process of pupation the non-digested content of the caterpillar hardens and forms the thin-walled, oval chambers in which the parasitic larvæ lie and in which they undergo their transformation into pupæ. The superficial layer is perfectly transparent, and at first is very flexible. Later, as drying occurs, it shrinks in on the walls of chambers and becomes hard and rigid, the whole forming the typical mummified carcass (Figs. 2, 4, 6). Practically all of the pupæ are oriented in a definite fashion in the carcass. Their heads are directed toward the anterior end of the carcass. Just before becoming immobile, the *Gnorimoschema* larva almost invariably turns the head upward in the gall chamber; likewise, the parasitic larvæ, just before pupating, orient themselves so that their heads are directed upward, in the direction of the anterior end of the carcass.

The imagines come out during the last week of August and the first week of September (Table I.). They escape by gnawing holes through the walls of the chambers and the superficial chitinous layer, both of which become very fragile. As a rule they all emerge practically at the same time. Several cases have been observed in which the entire brood has escaped within a period of ten minutes.

Once free from the carcass, they immediately gnaw a hole through the wall of the gall. Their escape is greatly facilitated by the habit of the caterpillar, just before becoming immobile, of eating out a passage-way to, or nearly to the epidermis of the plant. But in no case does the parasitized caterpillar secrete a silken plug. Hence, in order to escape to the exterior, the parasites have only to cut through the remaining thin portion of the wall.

The parasites must winter over in the imago state; otherwise

they would not be able to parasitize the normal or spring eggs of *Gnorimoschema*. Copulation, however, takes place immediately after the adults emerge, but the females do not parasitize the

TABLE II.

TABLE SHOWING VARIATION IN LENGTH OF LARVÆ IN THREE LOTS OF  
COPIDOSOMA.

Length in Lines.	Lots.			Length in Lines.	Lots.		
	I	II	III		I	II	III
1				29		3	3
2				30		10	7
3			2	31		3	1
4			3	32	I	5	
5			10	33		1	1
6		3	14	34		2	3
7			13	35		4	3
8		I	12	36		2	1
9			4	37		8	5
10		I	3	38		1	4
11		I	4	39	I	2	2
12			2	40	3	3	9
13			2	41	4	4	4
14			2	42	2	7	5
15	I		6	43	2	3	7
16	I		3	44	I	3	7
17	4	4	12	45	3	5	2
18		I	7	46	I	8	4
19	I	6	16	47	I	5	3
20		4	17	48	I	10	1
21			9	49	2	13	2
22		9	7	50		10	2
23		5	6	51		4	
24		3	4	52	2	8	
25		3	13	53		1	
26		3	5	54			
27		I	4	55	I	2	
28		4	I	56			

fall eggs of this moth. Only on one occasion has an attempt to oviposit in such eggs been observed. In this instance the few females which made the attempt were not able to penetrate the shell of the egg with the ovipositer.

#### 4. *The Abortive Embryos.*

One of the most interesting discoveries made in connection with the study of *Copidosoma* is what we shall call the abortive embryos or larvæ, to which brief reference has already been made. Abortive embryos occur in the development of many different species of both invertebrates and vertebrates. They

are especially common in mammals. For example, my colleague, Dr. C. G. Hartman, has found a great mortality of embryos in the development of the opossum. Degenerating embryos are found throughout the brief but entire period of gestation. Abortive embryos have been found in at least three other species which have a polyembryonic type of development. One of the two embryos which develop from a single egg of the earthworm, *Lumbricus trapezoides*, sometimes degenerates. Fernandez ('09) has observed rudimentary embryos in the South American armadillo, *Tatusia hybrida*, and I have on several occasions seen them in the blastocyst of *Tatusia novemcincta*. But in no case with which we are acquainted is their number and constancy of occurrence so striking as in *Copidosoma*.

Our attention was first attracted to these abortive embryos while dissecting out a batch of larvæ from a large caterpillar. Most of the larvæ in the lot were large and about on the point of undergoing pupation, but in addition to these large individuals, there were a number of smaller ones. At first it was supposed that two distinct species of parasitic larvæ were present, or that we had a condition similar to that described by Silvestri for *Litomastix*, of sexual and asexual larvæ. It was noted, however, that the small larvæ had the same general structure as the larger individuals, except that they still possessed the two involucre typical of all of the younger larvæ of this species.

A study of serial sections of more than a hundred polygerms has completely demonstrated beyond any possibility of doubt that the small rudimentary embryos are derived from the same egg as larger normal larvæ, and consequently do not belong to a different species. The sections show that degenerating embryos are to be found in every stage of development of the polygerm, from the time of the formation of single embryos until the larvæ are set free into the body cavity of the host. In Fig. 24 is shown a degenerating embryo which has not yet been completely cut off from its fellow by the constriction of the nucleated membrane. Its nuclei have already completely disintegrated. In Fig. 26 is another embryo well on the way to complete disintegration. Finally Fig. 17, which is a portion of a polygermal mass about at the close of dissociation, contains at least four or five rudimentary embryos. They stain darker than the normal individuals.

The degeneration of embryos or larvæ does not cease immediately after the dissociation of the polygermal mass, but such embryos are found up until the beginning of pupation. About fifty lots of free larvæ have been dissected out of caterpillars, and

TABLE III.

TABLE SHOWING THE NUMBER OF PARASITES IN FEMALE BROODS.

Brood.	No. of Individuals.	Brood.	No. of Individuals.
1	25	46	200
2	42	47	201
3	49	48	207
4	52	49	210
5	73	50	210
6	89	51	212
7	91	52	213
8	95	53	213
9	100	54	214
10	100	55	215
11	106	56	215
12	108	57	216
13	115	58	216
14	119	59	217
15	120	60	229
16	121	61	234
17	122	62	236
18	124	63	236
19	124	64	237
20	125	65	245
21	131	66	248
22	137	67	250
23	142	68	251
24	145	69	254
25	146	70	256
26	150	71	257
27	151	72	260
28	153	73	261
29	154	74	264
30	156	75	272
31	161	76	275
32	163	77	280
33	164	78	284
34	167	79	286
35	174	80	292
36	174	81	301
37	178	82	314
38	178	83	328
39	179	84	335
40	181	85	338
41	183	86	340
42	189	87	347
43	192	88	378
44	194	89	385
45	195	90	395

Total = 17,864.

Average = 198.48.



almost without exception degenerating individuals were found. During the early period of the free larval stage, any given lot will show great variation in the size of the larvæ. To show this, all of the individuals of three lots have been measured in the terms of lines on the eye-piece micrometer scale (Table II.). In Lot I. there were only thirty-two larvæ. All but six of these would have reached maturity. Lot II. contained 176 larvæ, but at least twenty of these were degenerating. Lot III. contained 257 larvæ, and probably more than a hundred of them would have degenerated eventually.

A series of sketches of these larvæ is shown in Fig. 25, *A* to *H*. The first four or five of these types would have developed to maturity, but such larvæ as those illustrated in *F* to *H* degenerate. The most common types of degenerating embryos are the small spherical or oval-shaped masses (*G*, *H*). In one extreme case the lot of embryos consisted of about thirty of these masses, together with only a single normal larva. Doubtless many other similar masses had already degenerated.

It is difficult to assign any definite cause to the degeneration of these embryos, although it probably has something to do with nutrition. In some cases it seems to be due to the fact that the division of the egg has been carried too far. Some of the primordia receive but few embryonic nuclei, and these are invariably the first to degenerate in the polygerm. In other cases the degeneration is apparently due to the lack of proper nutrition. Most of the polygerms are early surrounded by the thick layer of adipose tissue, upon which the early development of the embryos depends. But other polygerms are almost if not entirely barren of adipose cells, and it is an observed fact that the mortality of embryos in such cases is exceedingly high. In Fig. 14 one of these cases is shown. This polygerm, which is devoid of fat tissue, contains more than a hundred embryos, not more than thirty or thirty-five of which have developed normally.

#### V. NUMBER AND SEX OF COPIDOSOMA PARASITES FOUND IN GNORIMOSCHEMA.

The number of matured parasites developing in the *Gnorimoschema* larva has been determined in 162 cases. This has been



done by removing the carcass from the gall chamber a short time before the emergence of the parasites, and enclosing it in a small vial. After all of the parasites have emerged they are killed by filling the vial with 80 per cent. alcohol, and then counted under a binocular microscope. This procedure has the advantage of eliminating the possibility of contamination from other polyembryonic broods. Furthermore, the use of the binocular in counting enables one to distinguish readily the two sexes. The strong sexual dimorphism in *Copidosoma* makes this task rather easy. The females have the enlarged club-shaped,

TABLE IV.

TABLE SHOWING THE NUMBER OF PARASITES IN MALE BROODS.

Brood.	No. of Individuals.	Brood.	No. of Individuals.
1	41	32	178
2	53	33	179
3	61	34	180
4	67	35	180
5	90	36	180
6	93	37	182
7	96	38	190
8	100	39	190
9	101	40	192
10	106	41	199
11	107	42	199
12	113	43	202
13	118	44	204
14	119	45	214
15	124	46	215
16	124	47	218
17	124	48	223
18	127	49	225
19	128	50	232
20	136	51	233
21	137	52	236
22	138	53	236
23	139	54	245
24	142	55	247
25	147	56	272
26	152	57	277
27	168	58	278
28	171	59	323
29	172	60	324
30	177	61	328
31	177	62	345

Total = 19,874.

Average = 175.32.

terminal segment of the antenna, and bright yellow legs, while the males do not have the enlarged segment and the legs are of a

dark, more or less mottled color. One can therefore readily detect a mixed brood under the microscope.

The 162 broods studied were taken at random from the field, and therefore in all probability the data on numbers and sex yielded by them represent the approximate sex ratio for the species. These 162 broods contained a total of 31,001 individuals, or an average of over 191 to the brood. Ninety of these, or 55.56 per cent., contained only female parasites, 62, or 38.27 per cent., contained only male parasites, and 10, or 6.17 per cent., contained mixed broods of males and females.

There are therefore not only a larger number of female broods than male, but the average number of individuals in the former exceed that of the latter. Female broods average a little over 198 individuals to the brood (Table III.), while male broods average only about 175 (Table IV.). The range in the number of individuals in these broods (from 25 to 395 in the female, and from 41 to 345 in the male) makes it evident that these averages are of little significance, except, perhaps, to show that the fertilized egg is slightly more prolific than the unfertilized egg.

Of the total number of individuals (31,001), 63.41 per cent. are females and 36.59 per cent. males; but obviously the true sex ratio can not be based on these figures. It must be determined from the number of male and female broods. It would not be a difficult matter to determine this ratio were it not for the uncertainty of the origin of some broods. There is always the possibility in these insects that more than one parasitic egg has been laid in the egg of the host, and hence the parasites which later emerge may not constitute a true polyembryonic brood, but in fact represent two or even more such broods. Under the circumstances, the best that one can do is to determine approximately the sex ratio for the species. This can be done in the following manner. If we assume, as all previous workers have done, that each of the mixed broods is the product of at least two eggs, then, in accordance with the law of probability, we can determine the number of unmixed male and female broods, each of which must also have been produced from two eggs. Worked out on this basis, it is found that the ratio of females to males is 106/76 or a sex ratio of approximately 3 : 2

This leads to a discussion of mixed broods, and especially to a consideration of the question as to how such broods have come into existence. The obvious explanation of their origin is the one given above, viz., that they arise from two eggs. Marchal and Silvestri, who have studied the development of polyembryonic insects, both offer this explanation. They support the conclusion by citing the fact that two (or more) parasitic eggs are sometimes laid in the egg of the host. According to Marchal, such eggs develop independently, each producing a distinct polygerm and consequently a distinct brood. If the two eggs are of the same sex potentiality, the individuals developing from them will be either all females or all males, according to whether or not the eggs are fertilized or unfertilized. The dual origin of these double broods naturally elude detection in lots that have emerged. But if one of the two eggs is unfertilized and the other fertilized, the result will be a mixed brood, consisting of males and females. This conclusion of Marchal and Silvestri is strongly supported by the facts of polyembryonic développement in the armadillos, in which it has been conclusively demonstrated (Fernandez, '09, Patterson, '13) that all of the embryos of a given pregnancy are the product of a single egg. As a result, mixed litters are never found in these mammals.

That mixed broods may arise from two eggs in *Copidosoma* is supported by the fact that two polygerms are sometimes found in a single *Gnorimoschema* larva. However, certain facts concerning the condition of mixed broods in this species, make it doubtful whether the origin of all such broods can be explained in this obvious way. Careful dissections of something over a hundred parasitized *Gnorimoschema* larvæ have revealed only two cases in which a single larva contained more than one polygerm. Since 6.17 per cent. of all broods are mixed, and since a similar number of unmixed broods would have a dual origin, we should expect to find over 12 per cent. of all parasitized larvæ containing two polygerms, but instead, less than 2 per cent. are found.

Another line of evidence which is not in harmony with the view that mixed broods are always the product of two or more eggs, is the great preponderance of females in certain lots. Of

the nine complete lots (Broods 2 to 10) listed in Table V., the number of females in each case is greater than the number of males. In some cases (Broods 3, 4, 5, 7, 8), this difference is not so great but that the origin of each lot can be explained on the assumption that two eggs have been deposited in the egg of the host. But in Broods 2, 6, 9, and 10 the number of females in excess of males is indeed striking, making it difficult to explain the origin of such broods on the basis of two eggs.

In view of these facts, the writer is convinced that some other explanation must be offered for the origin of certain mixed broods; in fact, one involving the idea that a single fertilized egg may give rise to a few males as well as a relatively large number of females. This would be possible on the basis of the following assumption.

TABLE V.  
TABLE SHOWING THE NUMBER OF PARASITES IN MIXED BROODS.

Brood.	No. of Individuals.	Females.	Males.
1*	89	20	69
2	162	153	9
3	172	92	80
4	207	126	81
5	216	176	40
6	235	223	12
7	241	161	80
8	300	235	65
9	304	292	12
10	337	316	21
Totals. . . . .	2,263	1,794	469
Average. . . . .	226.3	179.4	46.9

\* This brood is not complete, owing to the fact that some of the larvæ and pupæ had been destroyed by a large dipterous larva.

If *Copidosoma* conforms to the general scheme for sex determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single

X chromosome. If such nuclei later divided in the typical manner and gave rise to embryos, such embryos would be males. One is encouraged to make this suggested explanation in the light of Bridges' ('13) discovery of the non-disjunction of the sex chromosomes in *Drosophila*. In *Copidosoma* the separation of the sex chromosomes during cleavage would be a case of "somatic" or "cleavage disjunction," while in *Drosophila* these chromosomes fail to separate or "disjoin" in the reduction division of the egg.

In conclusion attention should be directed to the frequency of *Copidosoma* in nature. At Woods Hole about twenty per cent. of all *Gnorimoschema* larvæ are infected with this parasite

TABLE VI.

TABLE SHOWING PERCENTAGE OF PARASITIZED CATERpillARS IN THE GALLS OF  
SOLIDAGO SEMPERVIRENS.

Number of Galls.	Date.	Parasitized by <i>Copidosoma</i> .	Normal Galls	Empty.	Parasitized by Other Parasites.
9	7-29-12	7	2	0	0
33	8- 5-12	5	15	0	13
33	8-17-12	9	16	5	3
56	8- 8-12	7	26	10	13
29	8-12-12	8	16	0	0
141	8-25-12	20	56	33	32
14	7- 7-13	1	13	0	0
16	7-14-13	0	13	0	0
39	7-15-13	8	31	0	0
38	7-19-13	4	*	*	*
23	7-23-13	2	20	0	1
38	7-26-13	6	*	*	*
27	8- 5-13	4	*	*	*
24	8-25-13	4	17	3	0
18	6-15-14	2	14	2	0
19	6-18-14	3	16	0	0
43	6-22-14	19	19	3	2
40	6-24-14	9	20	10	1
20	7-16-14	1	19	0	0
24	7-30-14	0	21	0	3
25	7-23-15	3	12	7	3
18	7-26-15	5	11	0	2
66	7-30-15	25	37	2	2
35	8- 4-15	14	35	1	6
Totals. . 828		166			

\* Record incomplete. About 20 per cent. of the caterpillars are parasitized by *Copidosoma*.

(Table VI.). As may be seen from the table, the extent of infection varies greatly in the lots of galls taken from different regions (those collected on a given date are all from a single locality). Plants which grow in exposed places, as along the

roadside or barren spots, carry a higher percentage of galls than do those which are located in protected regions. Likewise, the moth larvæ from the galls of the former are more highly parasitized.

#### SUMMARY.

1. *Copidosoma gelechiae*, which is a parasite in the Solidago Gall Moth, *Gnorimoschema salinaris*, has but one generation a year.

2. The egg of this parasite is probably laid during the month of May.

3. The type of development in *Copidosoma* is polyembryonic. The number of individuals average about 191 per brood.

4. In the youngest stages secured the process of division of the egg into embryonic primordia is already in progress. The young polygerm consists of two distinct regions: (1) An outer zone, or nucleated membrane, containing the free polar nuclei; (2) a central region, containing the true embryonic nuclei.

5. The embryonic nuclei segregate into groups, which become surrounded by a dense layer of granular protoplasm and form the primordia of the multiple embryos.

6. During early growth the polygerm elongates into a cylindrical-shaped structure, which becomes broken up into several spherical, primary masses by the formation of constrictions in the nucleated membrane. Each primary mass receives several of the primitive embryos.

7. The primary masses become broken up into secondary masses by further constrictions of the nucleated membrane. At the end of these divisions, each embryo is separated from the others and is surrounded by an inner and an outer involucre—the former derived from the granular protoplasm and the latter from a portion of the nucleated membrane.

8. The interstices between these masses become filled with an inter-embryonal substance derived from at least three sources: elements from the nucleated membrane, leucocytes, and cells from the adipose tissue, which usually is laid down in the form of a thick layer on the outer surface of the polygerm. The entire structure thus becomes a complex, which may be called the polygermal mass.



9. The dissociation of the inter-embryonal substance sets the larvæ free in the body cavity of the host. This occurs during the last week of July.

10. Abortive or degenerating embryos are found throughout the entire period covered by the polygerm and free larval stages.

11. The free larvæ destroy the entire contents of the caterpillar, except the chitinous parts of the tracheæ, and leave only the superficial layer of chitin of the body wall intact. The detritus left in the larval chitin hardens to form thin-walled, oval chambers in which the larvæ lie and undergo pupation. The superficial layer of chitin also hardens, and the larval skin thus becomes transformed into the typical mummified carcass, filled with the parasitic pupæ.

13. Pupation takes place during the first ten days of August and lasts about a month.

14. The number of adult parasites emerging from the carcasses varies from 25 to 395. There is a preponderance of females, about 55 per cent. of all broods being females. Furthermore, the average number of females emerging from a single carcass is 198 as compared with 175 for the males. Ten mixed broods of males and females have been obtained. Some of these have doubtless arisen from two or more eggs; but it is suggested that such broods may also arise from a single fertilized egg, by a process of disjunction of the sex chromosomes during the early cleavage stages.

WOODS HOLE, MASS.,

August 12, 1915.

#### LITERATURE REFERENCES.

Bridges, C. B.

- '13 Non-disjunction of the Sex Chromosomes of *Drosophila*. Jour. of Exp. Zool., Vol. XV., pp. 587-606.

Fernandez, M.

- '09 Beiträge zur Embryologie der Gurteltiere. Morpholog. Jahrb., Bd. 39, pp. 302-333.

Howard, L. O.

- '85 Description of North American Chalcididæ. Bureau of Entomology Bulletin.

Marchal, P.

- '98 Un exemple de dissociation de l'oeuf. La cycle de l'*Encyrtus fuscicollis*. C. R. Soc. Biol. Paris. T. 5. pp. 238-240.

Marchal, P.

- '04 Recherches sur la Biologie et le Developpement des Hymenopteres Parasites.



I. La Polyembryonie Specifique ou Germinogonie. Arch. de Zool. Exper. et Gen., Vol. II., pp. 257-335.

Martin, F.

- '14 Zur Entwicklungsgeschichte des polyembryonalen Chalcidiers Ageniaspis (Encyrtus) fuscicollis. Zeit. f. Wiss. Zool., Bd. 110, pp. 419-479.

Patterson, J. T.

- '13 Polyembryonic Development in Tatusia novemcincta. Jour. Morph., Vol. 24, pp. 559-684.

Riley, C. V.

- '69 The Solidago Gall Moth. First Annual Report of the State Entomologist of Missouri, pp. 172-178.

Sacken, Baron Osten.

- '63 Lasioptera Reared from a Gall on the Goldenrod. Proceed. of the Ent. Soc. of Phil. Vol. 1, pp. 368-370.

Silvestri, F.

- '06 Contribuzioni alla conoscenza biologica degli Immenotteri Parassiti. I. Biologia del Litomastix truncatellus Ann. d. Regia Scuola Superiore di Agricoltura di Portici, Vol. VI., pp. 1-51.

Silvestri, F.

- '08 Contribuzioni alla conoscenza biologica degli Immenotteri Parassiti. II. Sviluppo dell'Ageniaspis fuscicollis. Ibid., Vol. VIII., pp. 1-27.

Wheeler, W. M.

- '10 The Effects of Parasitic and other Kinds of Castration in Insects. Jour. of Exper. Zool., Vol. 8, pp. 377-438.



## DESCRIPTION OF PLATES.

## PLATE I.

FIG. 1. A typical gall of *Gnorimoschema salinaris*, Busck, situated at the base of the stalk of the swamp goldenrod, *Solidago sempervirens*.  $\times \frac{1}{4}$ .

FIG. 2. Gall cut open to show the position of the mummified carcass of *Gnorimoschema*. Natural size.

FIG. 3. Gall cut open and carcass removed to show the shape of cavity. Note that the walls of the cavity are smooth and that the excrement from the caterpillar is packed in the bottom of the cavity. Natural size.

FIG. 4. Mummified carcass from gall shown in Fig. 3. Natural size.

FIG. 5. Lepidopterous larva which is an inquiline in the gall of *Gnorimoschema*. Note the irregular shape of the cavity which contains scattered trash and excrement. Natural size.

FIG. 6. This gall shows an incomplete passage-way, lying just above the head of the carcass. Normal size.

FIG. 7. Side view of a gall showing the orifice of the passage-way, closed by silken plug. Natural size.

FIG. 8. Stalk of swamp goldenrod containing two galls.  $\times \frac{1}{4}$ .

FIG. 9. Gall containing a non-parasitized caterpillar. Natural size.

FIG. 10. Gall containing a parasitized caterpillar. Natural size.



1



2



3



4



5



8



6



7



9



10





## PLATE II.

FIG. 11. Photomicrograph of a section of an irregular polygermal mass.  $\times 40$ .

FIG. 12. Photomicrograph of a single embryo from mass shown in next figure.  $\times 180$ .

FIG. 13. Photomicrograph of a longitudinal section of a flat, plate-like polygermal mass.  $\times 40$ .

FIG. 14. Photomicrograph of a spherical polygermal mass which is barren of adipose tissue.  $\times 40$ .

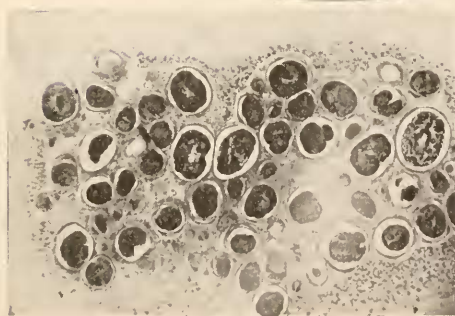




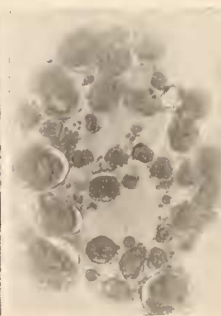
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## PLATE III.

FIG. 15. Photomicrograph of the middle portion of longitudinal section of a small caterpillar. A fat body containing a polygerm lies just below the intestine.  $\times 44$ .

FIG. 16. Photomicrograph of a portion of a section of a polygermal mass which was about to begin disintegration.  $\times 44$ .

FIG. 17. Photomicrograph of a section of a polygermal mass undergoing dissociation.  $\times 44$ .

FIG. 18. Photomicrograph of a mass of free larvae from the body cavity of the caterpillar. Note that each embryo is still surrounded by the involucre.  $\times 44$ .

*Reference Letters Used in Plates IV.-VI.*

*A.E.*, Abortive Embryo.

*A.T.*, Adipose Tissue.

*C.*, Clear space left by contraction of  
Precipitated Material.

*E.N.*, Embryonic Nuclei.

*G.L.*, Granular Layer.

*I.I.*, Inner Involucre.

*I.S.*, Inter-embryonal Substance.

*N.M.*, Nucleated Membrane.

*O.I.*, Outer Involucre.

*P.D.*, Primary Division of polygerm.

*P.E.*, Primitive Embryo.

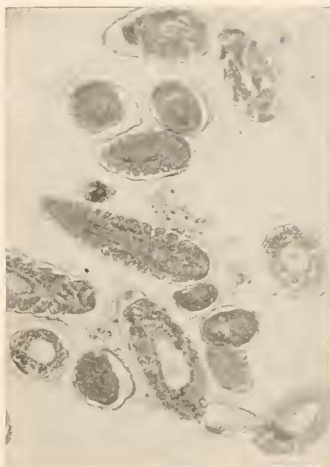
*P.M.*, Precipitated Material.



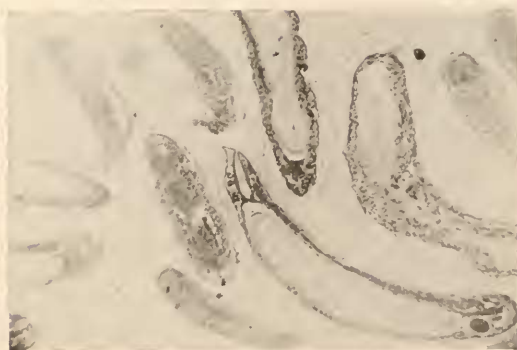
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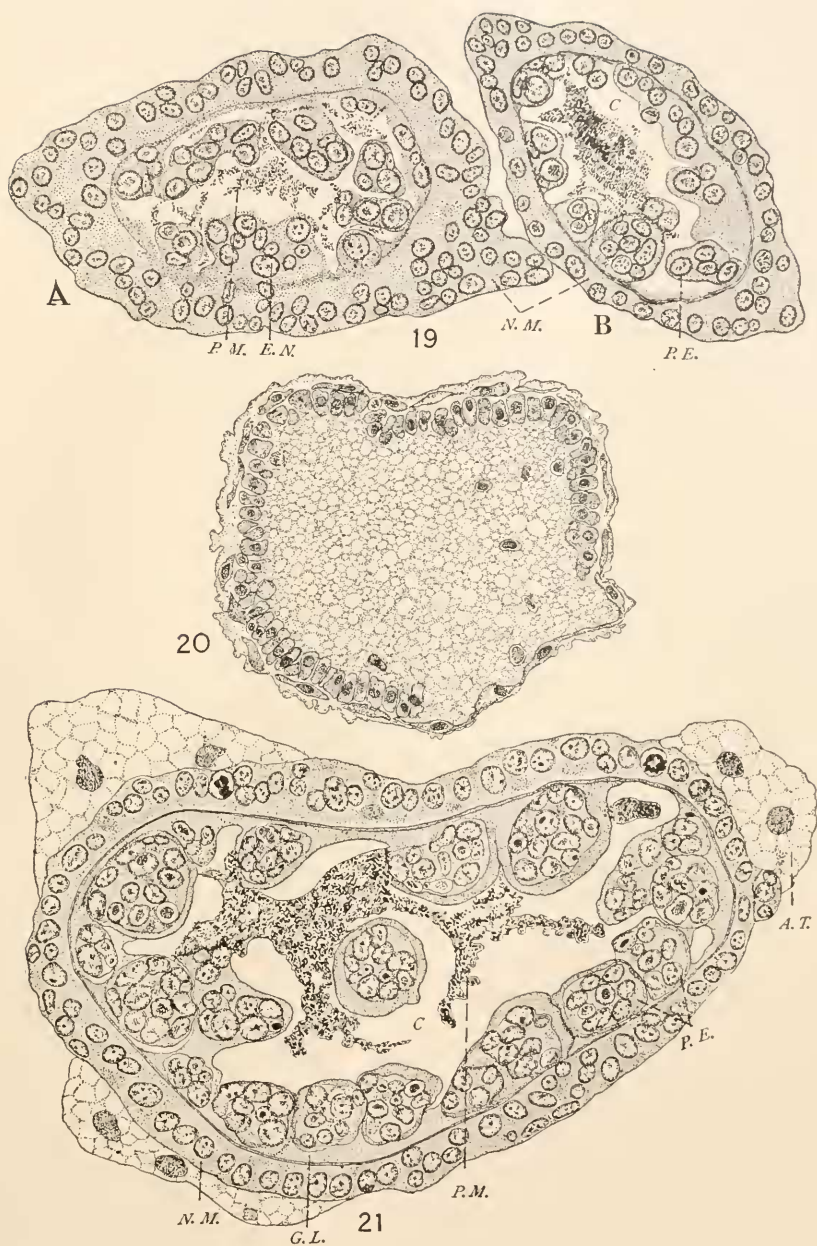


## PLATE IV.

FIG. 19. *A* and *B* longitudinal sections of two polygerms lying close together in the same caterpillar. These polygerms show an early phase of the segregation of the embryonic nuclei to form the separate embryos.  $\times 489$ .

FIG. 20. Section of an egg of *Gnorimoschema* which has started to develop parthenogenetically.  $\times 173$ .

FIG. 21. Longitudinal section of a polygerm showing the end phase of embryo formation.  $\times 480$ .







## PLATE V.

FIG. 22. One end of a longitudinal section of a polygerm showing three of the twelve primary divisions into which it has been divided by constrictions of the nucleated membrane.  $\times 373$ .

FIG. 23. Section of a primary mass showing the process by which it is further divided up into secondary masses by constrictions of the nucleated membrane.  $\times 508$ .

FIG. 24. Section of a single isolated, primary mass about at the close of its division into single embryonic masses.  $\times 257$ .





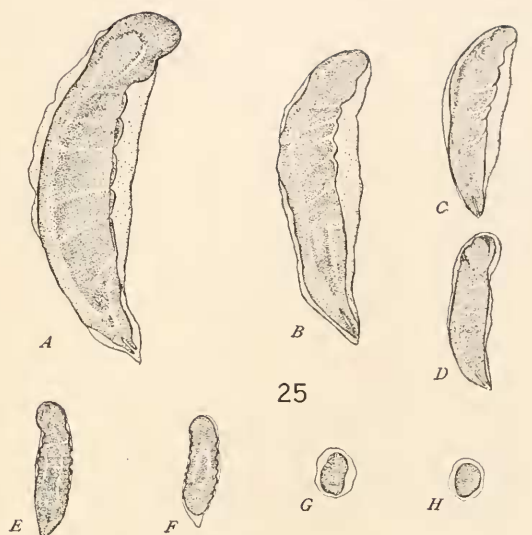




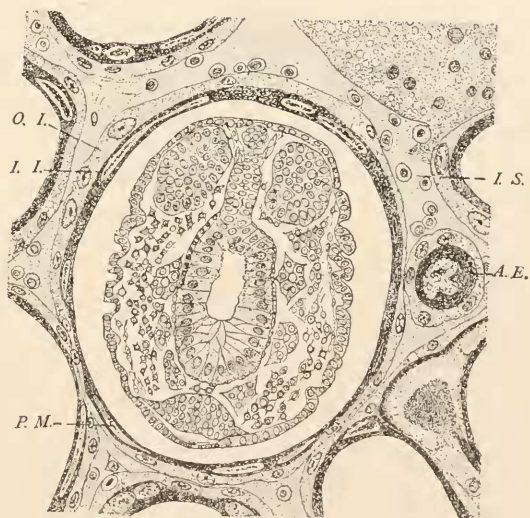
## PLATE VI.

FIG. 25. *A* to *H*, Series of sketches from Lot III of the free larvae listed in Table III. This figure shows the great variation in size of the larvae from a single caterpillar. They are all drawn to the same scale.

FIG. 26. Detailed drawing of a section of one of the embryos seen in Fig. 13. It shows the relation of the inter-embryonal substance and involucres to the embryo.  $\times 187$ .



25



26



## DISTRIBUTION OF FOLLICULINA IN 1914.

E. A. ANDREWS.

The finding of vast hordes of the *Stentor*-like infusorian *Folliculina* both in 1912 and 1913 throughout the whole extent of the Severn River which is a brackish side branch of the Chesapeake Bay, led to further examination in 1914 to see if this were a phenomenon to be repeated annually or only a rare inroad of an outside fauna into new territory.

In 1913<sup>1</sup> *Folliculina* was found in inconceivable numbers living upon the leaves of the fresh water plants *Elodea* and *Potamogeton*, which have taken possession of definite zones of shallow brackish water along some fifty and more miles of extent of the river and its side creeks. It was also found on *Elodea* in Whitehall River, just to the north of the Severn.

In 1914 it was taken on *Elodea* from the head of the Magothy River, August 13, and on floating *Elodea* in the mouth of the Magothy, August 23, when it was also found living upon stunted *Elodea* growing in the narrow inlet canal to the nearly shut off side branch known as the Little Magothy. It was taken also at Deep Creek, a side branch of the Magothy.

As the Magothy opens into the Chesapeake some seven miles from the Severn, the distribution of *Folliculina* is quite extensive. Moreover, in 1880 Ryder<sup>2</sup> found *Folliculina* in great numbers upon oyster shells in shallow water on the west coast of the Chesapeake, and as he seems to have then been at St. Jerome, St. Mary's County, which is sixty miles down the Bay from the Severn, the distribution of *Folliculina* is known for side branches of the Bay opening into it seventy miles apart, approximately.

It is to be expected then that exceedingly large areas of the side waters of the Chesapeake may be inhabited by this little-known protozoan, which in the mid-summer season adds greatly

<sup>1</sup> See BIOL. BULL., XXVI., No. 4, April, 1914.

<sup>2</sup> *Am. Nat.*, 14, 1880.

to the plankton, or swimming fauna, as well as to the microscopic life attached to the summer vegetation of these waters.

Its advent and departure in Chase's Creek, a branch of the Severn, showed in 1914 even more suddenness than in 1913, while its time of abundance was noticeably less though actual numbers present were even more vast.

Though searched for from the middle of June, every few days, *Folliculina* was found first on July 19, 1914. It then appeared only here and there, not on every plant of *Elodea* and on very few plants of *Potamogeton*. On the sprays of *Elodea* the *Folliculina* showed on comparatively few leaves, like black soot stuck on the leaves; both isolated individuals and aggregates occurred



FIG. 1. Leaf of *Potamogeton* showing scattered colonies of *Folliculina*.  $\times 3$  diam. Photograph of preserved specimen.

but there were very few large aggregates covering half the surface of a single leaf. Most leaves had none, some leaves had many scattered individuals. On the stems there were noticeable numbers of the small form of sac. The occurrence on leaves seemed entirely arbitrary as if from settlements of swimmers: the *Folliculina* was not now crowded toward the tips of the sprays but scattered along many inches of the spray.

At the date of this first appearance, jellyfish had been common for two weeks but the other conspicuous summer visitor to these waters, the young menhaden now for the first time came along the shores over the *Elodea*, which may be correlated with the

feeding of the menhaden upon plankton in which the free swimming *Folliculina* may be included as possible food for the menhaden.

At this date the *Elodea* had grown up to a height of twenty inches and formed some flower stalks and buds at the surface, so that there had been a long period in which suitable attachment base for *Folliculina* was present but the *Folliculina* had been absent.

July 21 the water after long drought was turbid from the presence of plankton and the *Folliculina* had increased but little, appearing as black spots on one out of several hundred sprays of *Elodea* and one out of many thousands of *Potamogeton* sprays. Only a few of the leaves on each inhabited spray had dense aggregates, so that the question arises: why do the *Folliculina*



FIG. 2. Tip of leaf of *Elodea* covered with a colony of *Folliculina*.  $\times 15$  diam. Photograph of preserved specimen.

crowd together in these rare, isolated aggregates? When sprays of these dates were put into aquaria they gave rise to free swimming forms, thus showing that these early settlers need not remain fixed but might contribute to additional distributions.

On July 27 *Folliculina* had become much more abundant upon sprays of *Elodea* and *Potamogeton*; some of the free-floating fragments on the surface appeared black with the accumulated



*Folliculina*. In the water also some free-swimming *Folliculina* could be seen near the surface swimming all through the water as well as close to floating plants.

Out in the Severn River a two-quart jar of water taken up at random at the surface showed several free-swimming *Folliculina*;



FIG. 3. Photograph of a preserved colony that had been formed on surface of the water in aquarium; showing form of case and tube spirals as well as animal retracted within case. Enlarged 30 diameters.

three days later these had settled down on the side of the jar and were in two groups, two individuals in one and five in the other, so that at least seven were in the two quarts of surface water, which would make an immense number for the entire river.

By August 1 much of the *Elodea* growing in the *Elodea* zone along shore was black with aggregates of *Folliculina*. Free swimmers were in the water of the creek in vast numbers: a quart dipped from the surface at random showed in a white bowl from fourteen to one hundred, by actual count, for each quart of water from the surface. By drawing the bowl along the surface, the *Folliculina* swimming free were concentrated till thousands in a quart made it dark as if sprinkled with black pepper. Though these free-swimming *Folliculina*s easily escape notice in the greenish water turbid with plankton and sediment, they are readily observed in calm water by an eye near the surface; and standing in water five feet deep one may see them swimming



FIG. 4. Photograph of two young colonies of free swimmers that have just settled on surface of water in aquarium and formed sacs but no tubes: one individual on extreme left is still in motile form. Preserved specimen,  $\times 20$  diam.

rapidly in all directions, individually in straight and in curved paths. Many deep down in the water were seen best by holding a white object below them, but most of them were near the surface where they congregated especially about any floating object as fallen leaf or floating chip, seemingly influenced by its presence so that they swam toward it.

While at this time the *Folliculina* continued to colonize the new growths at tip of the *Elodea* as fast as it grew so that the

black aggregates crowded on the young leaves nearly to the tip where only the newest leaves were as yet unoccupied; by August 18 the extension of the *Folliculina* hosts had ceased. The tips of the growing *Elodea* were now bare or free from *Folliculina* back some twenty leaves from the tip and many of the old dwellings on the lower leaves were deserted. These dense black colonies on old leaves contained in fact but few living *Folliculinas*.



FIG. 5. Photograph of natural size sprays of *Elodea* preserved to show successive phases of colonization in 1914. Spray on left has grown enough to form flower but as yet but a very few isolated individual *Folliculina* have settled upon it. The next spray shows scattered tubes all along its length. The third spray shows dense aggregations of colonies even up to the tips of the rapidly unfolding new leaves. The fourth spray illustrates the subsidence in colonization: the new colonies no longer cover the leaves at the tip of the spray but these grow more rapidly than the new colonists occupy them and are left more nearly free from any *Folliculinas*.

By August 26 this falling off in the colonization and rapid disappearance of *Folliculina* was most pronounced: the *Elodea* sprays showed an abrupt transition from the lower leaves black from dense population of tubes, for the most part empty, to the upper leaves only sparsely inhabited with scattered individuals. Evidently some sudden change had operated not only to check the previously rapid spread of the *Folliculinas* onto new leaves but to

almost exterminate them. Yet many remained alive here and there so that when large quantities of the *Elodea* were put into aquaria many free swimmers escaped. Yet these after forming new tubes on the surface of the water did not remain alive but had all vanished September 5, though in such apparently normal environment others had been kept two weeks in captivity earlier in the season.

Thus while appearing after the middle of July and being extraordinarily abundant in August, the *Folliculina* were all gone about the end of August and no way was found of keeping them longer. Their period of existence in accessible regions of the river was scarcely six weeks.

In 1913 they appeared before the end of June and a few lingered on to the first of September in nature and were kept in aquaria in a warm room till the 27th and a few till November 11.

In 1912 no live ones were found after September 8. This enormous crowding of the waters with free-swimming *Folliculina* and dense settlements of the case-making *Folliculinas* during about a month, the last weeks of July and the first of August, coincides with very high temperatures and abundance of microscopic plankton in these waters but it is not at all evident either why the *Folliculinas* should not come earlier, as they did in 1913, or remain later as they did in 1913 and 1912.

The great rapidity of their colonization of large areas suggests either very great immigration or else very rapid multiplication, or combination of both. As all material searched in the daytime in 1913 failed to show more than a few cases of multiplication, most all the free-swimming forms being merely the case-making forms again freed, material was collected at all times of the night in 1914, but here again but few cases of division were observed.

Hence it seems unlikely that fission of a few immigrants actually produced the vast numbers found on the leaves of plants, and it is probable that very large numbers came into the river suddenly from some outside source and these settling down, migrating out again, and in some cases increasing by fission, gave rise to the succession of dwellings covering the leaves for some two months.

The causes leading to the immigration as well as the causes of rather sudden diminution of numbers and utter disappearance remain entirely unknown.

The food of the case-inhabiting *Folliculina* being bacteria and some larger forms of plankton, the disappearance of *Folliculina* may well be associated with changes in food supply, in turn brought about in connection with such changes as those of temperature and salinity.

The motile forms take no food and may be enabled to settle and to continue migration and multiplication only when feeding conditions allow the sessile form to accumulate enough energy.

#### SUMMARY.

1. The vast swarms of swimming protozoans of the genus *Folliculina* that were found to settle down over the aquatic plants along the shores of side branches of the Chesapeake Bay in 1912 and 1913, came in even greater numbers in 1914, and it is therefore probable that this immigration and colonization is a regular annual phenomenon.

2. The incursions of swimming *Folliculina* do not take place as soon as the plants have grown enough to supply places for attachment, and the departure or disappearance of the living *Folliculinas* antedates the cessation of growth and final dying down of the plants upon which they settle.

3. As far as evidence is available the numbers that crowd the leaves arise more from immigration from without the area than from division of animals that have already settled in the area.

4. The times of appearance and disappearance differ in successive years.

5. It is suggested that conditions of food possibilities are determining factors in these inroads into the brackish fauna.

6. The great number of free swimming forms makes them, for the time being, an important factor in the plankton.

7. The crowding of the dwellings or cases on the leaves all along the shores is a considerable element in the transformation of matter which, arising from decay of organic materials, is transformed into bacteria and other plankton organisms, which in turn are eaten by *Folliculina* and enable them to secrete resisting tubes and sacs which finally settle to the bottom of the river.

## PHENOMENA OF ORIENTATION EXHIBITED BY EPHEMERIDÆ.<sup>1</sup>

F. H. KRECKER.

It is a well-known fact that in alighting Ephemeridæ orient positively to a breeze. I became interested in this reaction and the observations made naturally lead to others on reactions to gravity and to light, and to the results of a conflict between any of these three stimuli.

The observations were made during the summer of 1915 at the Lake Laboratory of Ohio State University at Cedar Point on Lake Erie. Ephemeridæ appear here in almost incredible numbers. When a brood is at its height it is a very common occurrence to find piles of the insects three or four feet square and six to eight inches deep under electric lights. At a neighboring amusement resort several carts were required each morning to haul away the dead insects. The species with which the following observations are especially concerned is *Hexagenia variabilis*. The number, variety and arrangement of lights at the resort presented favorable conditions for observing the reactions to light of great numbers of individuals in what may be termed natural surroundings. The equipment used for experiments with air currents and gravity was simple and largely improvised. Nevertheless, since it is not primarily my purpose to measure intensity of stimuli or rapidity of reaction, I believe the results obtained have some interest and value.

### REACTIONS TO A CURRENT OF AIR.

There was a question in my mind as to whether the positive orientation of the Ephemeridæ to a breeze is a response to the breeze per se or whether other factors are concerned. In order to test this I took a piece of glass tubing several inches long and sent through it a weak but steady current of air so directed

<sup>1</sup> Contribution from the Department of Zoology and Entomology, Ohio State University, No. 43.



as to strike the insects on the side of the body. They were resting on boards placed horizontally. A few of them flew away but most of them eventually faced the current. Individuals placed on a rough surface, such as a wire screen, which afforded a better foothold frequently tried to walk away. When facing the current of air an individual would raise its long, slender front pair of legs and extend them forward and upward at an angle of about 40 degrees. When held in this way the legs resemble antennae and it is possible they have a sensory function. However, cutting them off had no apparent effect on the reactions here in question. The time required for the turning reaction varied from an almost instantaneous response to two minutes. In the majority of cases the response was gradual and occupied from 30 seconds to one minute. The rapidity of reaction depended upon a correlation between the strength of the breeze and the part of the body it struck.

The influence of the area stimulated is shown in experiments with the wings. The latter are large in proportion to the body and meet over the back in a perpendicular position. They, therefore, present quite a broad surface. When a current of air of an intensity sufficient to blow the wings slightly to one side was directed against them individuals would react in fifteen to thirty seconds, whereas when this current was directed against the thorax or the abdomen the response was slower, if indeed any occurred. A stronger current directed against any of these parts brought about a correspondingly more rapid reaction.

In another series of experiments a current of air was directed from the posterior lengthwise of the body along the dorsal surface of a number of individuals. The response in these circumstances was also an eventual facing about to the current. A current of air striking an individual longitudinally along the mid-dorsal surface is neutral so far as lateral directions are concerned. In the cases here in question the current blew the wings to one side or the other and then as before the insects turned around toward the side on which the strain was exerted.

The experiments were repeated on a group of individuals from which the wings had been removed. The results from a current of air striking the insects on the side of the body were the same



as before; the insects faced the current. However, when a current was directed from the rear longitudinally along the dorsal surface of the body the previous results were not repeated. In some cases the insects crawled with the current and away from the point of origin. In other cases they remained stationary and took an attitude similar to that assumed when facing the current. If the current became very strong they either attempted to crawl away or they retained the attitude until blown off their feet. When the current veered sufficiently to strike them on the side they began to turn toward it.

In these experiments with air currents the first noticeable response from the insects was an attempt to hold on to the surface upon which they were resting. This they did by fastening their claws firmly and even changing the position of the legs. When the current became so strong as to make it difficult to remain attached and especially when the body was blown over to one side the insects began to change position, rather hesitatingly it appeared, and to face about toward the direction from which the current came. When an insect reached a position where it did not seem to have difficulty in maintaining its hold it came to rest. This usually meant that it was directly facing the current, although sometimes it stopped at a point between a half and a complete about face. A half about face could generally be made complete by increasing the strength of the current.

When directly facing a current of air an individual is in the optimum position for resistance; it presents the least surface and the claws because of their backward curve have the maximum effect in holding the body. On the other hand when an individual stands sidewise to the current a greater surface is presented, the claws are not in a relatively favorable position and attachment is clearly more difficult. With regard to the more rapid reactions which result when the current strikes the wings it may be said that the proportionately great expanse of the wings above the body's center of gravity gives them such a leverage that the body is more easily tipped over, a strain is more quickly felt and attachment more quickly made difficult. In those cases in which a current struck wingless individuals from the posterior there was practically no obstruction to the current

and it consequently did not so easily cause strain or seriously disturb the attachment and there was therefore no turning reaction.

It would appear from the foregoing experiments that the Ephemeridæ do not change position under the stimulus of a breeze until a strain is exerted on the organs of attachment. That this does not merely mean that the response was delayed, until a breeze of a given intensity developed is shown by the fact that a comparatively weak breeze directed against the wings alone had the same effect as was caused by a somewhat stronger breeze against the thorax. There is, therefore, evidence, I believe, for concluding that Ephemeridæ do not orient positively to a breeze because of sensations derived from the breeze per se but that they react positively to tension exerted on the muscles of attachment.

#### REACTIONS TO GRAVITY.

The position of Ephemeridæ when resting upon a perpendicular surface is negative with regard to the earth's surface and usually approximately vertical to it, although variations as great as 45 degrees occur. On comparatively smooth surfaces the orientation is more generally an approximation to the vertical, whereas on surfaces such as a wire screen, which affords a good foothold at any angle, variations from the vertical may occur in 50 per cent. of the individuals concerned. Individuals picked up by the wings and replaced head downward, if they are not so disturbed as to fly away, will struggle to gain a foothold. The position of the claws, which are adapted to a vertical position, make attachment rather difficult. This difficulty is increased by the fact that the long abdomen is thrown forward and downward and thus tends to destroy equilibrium. On comparatively smooth surfaces such as a planed board the insects rarely succeeded in maintaining their equilibrium long enough to gain a footing. On a wire screen they were more often successful and once they gained a footing and their equilibrium they retained the new position. The picking up process caused so many of the insects to fly away that other methods were tried. Several individuals were placed in a vertical position on a straw hat held perpendicularly and then the hat was slowly revolved until the

insects were upside down. The overhanging abdomen disturbed the equilibrium of some of them sufficiently to cause them to lose their hold and fly off. The others retained their footing, in some cases by changing the position of the legs, and remained in the inverted position for ten to fifteen minutes which was as long as they were watched.

In explanation of the position normally assumed on an upright surface the evidence derived from the experiments seems to indicate that the position taken is not a negative reaction to gravity per se but that it is largely, if not entirely, due to the character of the insect's means of attachment.

Results obtained from experiments performed to test the influence of a breeze upon the position of the insects on a perpendicular surface support this view. A current of air was directed against the side of individuals resting in the normal upright position on a perpendicular surface. As they turned the current was so directed as to bring them still further around. During the process some of them could not retain their foothold and flew off. The others turned completely around and faced directly downward. They maintained the inverted position at least as long as they were under observation, ten to fifteen minutes, which length of time, in view of a constant coming and going among those normally situated, seemed sufficient.

#### REACTION TO LIGHT.

The conclusions with regard to reactions of the Ephemeridæ to light are largely the result of observations made in the amusement resort already mentioned. The observations have to do mostly with artificial light. The insects react negatively to bright sunlight and seek the shade. They are strongly attracted to the lighter colors of artificial light. In the resort there are a great many electric lights of sixteen candle power intensity with colorless glass bulbs. Many of them are attached in a horizontal position to the sides of buildings in such a way that there is a perpendicular surface either above or below them and frequently on all sides.

The reaction to these lights seems to be satisfied if the insects can come to rest within a zone which begins approximately six inches from the light and covers a radius extending outward for

about twenty-four to thirty inches. When individuals enter this optimum zone they alight, if a surface is available, and orient themselves in such a way that the body is parallel with a radius projecting from the light. After alighting the insects usually remain at rest, although there may be a certain amount of crawling toward a position nearer the center. This is more often done by those nearer the outer limits of the zone. When the insects are numerous they become arranged in rows consisting of individuals either directly behind one another or slightly to one side and they thus form a striking pattern of radiating lines.

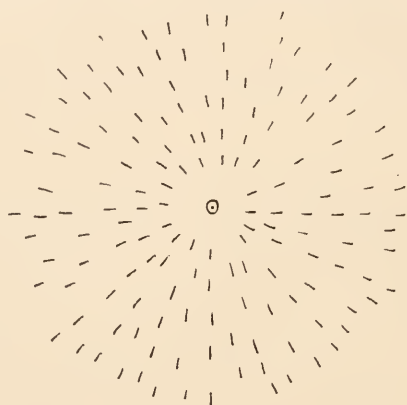


FIG. I.

The accompanying figures illustrate the positions assumed with regard to lights in different positions and combinations.

The first figure illustrates the position assumed when the surface extends about a light in all directions whether the plane be horizontal or vertical. When any portion of the surface is absent the pattern is of course interrupted to a corresponding extent. The clear zone immediately surrounding the light was approximately six inches wide. I shall call it the excitement zone. Individuals that entered this zone became greatly excited and fluttered about the light in a confused state. There was no evidence to show that individuals at rest deliberately entered the excitement zone. Those immediately bordering on it were rather restless and occasionally in crawling about some were pushed into it and others on taking wing came within the influence of the light.

The second figure shows lights arranged along the lower edge of a perpendicular surface at intervals of twelve to fifteen inches. About each light was the usual excitement zone and upward from this extended the radiating lines of insects in the optimum zone. As shown in the diagram these lines were rarely at an angle of less than 35 degrees. This was due to the fact that below this point the lines from neighboring lights conflicted and caused such confusion among the insects as to obliterate regular alignment. The greatest confusion occurred in the comparatively short space



FIG. 2.

between the lights where insects attempting to arrange themselves about one light constantly came into conflict with others attracted to the neighboring light.

When the insects rested on a horizontal plane about a light they faced it. The most striking feature connected with the arrangement of the insects on a perpendicular surface was that the individuals on opposite sides of a horizontal plane passing through the center of a light had opposite ends of the body directed toward the light. The insects below the plane or parallel with it faced the light, whereas those that were above the plane were turned away from the light. In other words all the insects, except those parallel with the horizontal plane, approximated a vertical position with the anterior end uppermost. Those above the plane and with the posterior end directed toward the light were apparently as well content as those below the plane and facing the light.

The position of the insects on a horizontal surface shows that other things being equal they face the light. It is reasonable to conclude that their normal reaction to light is positive. The negative position assumed on a perpendicular surface above a light can be explained, in view of the air current and the inversion experiments, as being due to the difficulty experienced in maintaining a foothold in the inverted position.

Some observations were also made on the relative influence of white and colored lights. On the sides of one of the buildings in the resort there was a succession of alternating white, red and blue lights. The slightly yellowish white bulb attracted the insects in greatest numbers. There was the usual excitement zone and the regular alignment of those at rest. The number of insects about the red and the blue bulbs was decidedly small and as between the two lights about the same. These lights appeared to have a quieting effect on the insects. The alignment was similar to that described for white lights but there was no well-defined excitement zone, in fact the insects crawled about the bulbs without exhibiting markedly abnormal reactions.

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## CELL MULTIPLICATION IN THE SUB-CUTICULA OF *DILEPIS SCOLECINA*.<sup>1</sup>

DALTON G. PAXMAN.

### INTRODUCTION.

The process of cell division in cestodes as compared with that in other Metazoa is apparently quite abnormal. An examination of cestode material at once reveals the fact that mitotic figures are very rare, and that an explanation of the process of cell division analogous to any of the common types is apparently impossible. The opinion of the various workers in cestode cytology, as to how cell division is taking place, varies greatly. Some state that it occurs by mitosis, others by amitosis, while it has been asserted that nuclei arise 'de novo' from the cytoplasm.

Child ('07) noted the apparent infrequency or total absence of any evidence of mitosis in *Moniezia*, even in regions where rapid growth was taking place. He says, "If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. And in the somatic cells of the adult body it appears to be the usual method at all times."

Young ('08), working with *Cysticercus pisiformis* describes what he calls the "de novo" formation of cells. He observed irregular masses of coarsely granular cytoplasm lying in the meshes of the parenchyma network. These masses contain numerous small deep staining granules scattered haphazard through the mass. Shortly succeeding the formation of these granules, a nuclear membrane is formed around them; the newly formed nucleus, together with a small mass of cytoplasm, becomes partly constricted from the parent mass; and the daughter cell has been formed."

Further, he says: "I believe that the nucleus in these forms is not a morphological, but a physiological entity; that the

<sup>1</sup> A thesis presented to the graduate faculty of the University of North Dakota in partial fulfilment of the requirements for a master's degree.



nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that the granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being thus occasionally converted into a nucleus; and the nucleus varies in structure from time to time in response to the varying physiological demands made upon it. . . . Further if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible, a special vehicle for the transference of hereditary qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyma, muscle, nerve, etc.—must be determined by physiological causes alone.”

Richards (1911), working with *Moniezia*, does not agree with Child. He says (p. 158): “I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis. Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonia and cleavage divisions are accomplished.”

Mary T. Harman ('13, p. 223) states: “My observations have not shown that amitosis does not take place in *Taenia* or *Moniezia*, but they have shown no condition which cannot be as readily explained as the result of mitotic as of amitotic division.”

#### MATERIALS AND PROCEDURE.

The form I worked with was *Dilepis scolecina* parasitic in the small intestine of the double-crested cormorant (*Phalacrocorax dilophus*). These birds are found abundantly near the shores and on the islands of Devils Lake, North Dakota.

Immediately after the bird was killed, the cestodes were removed from the intestine and placed in fixing solution. Flemming's solution and cestode mixture were the fixatives used. Flemming's solution blackened the tissue so that the results

from it were not satisfactory. The cestode mixture, however, gave excellent results.

The stains used were the following: Heidenhain's iron-alum-haematoxylin without counterstain; safranin counterstained with light green; thionin counterstained with acid fuchsin; methyl green counterstained with acid fuchsin; and safranin counterstained with water blue.

#### OBSERVATIONS.

I began my study of cell multiplication in cestodes without any previous knowledge of what had been done in the field of cestode cytology. Moreover, I completed the study of my material and drew my conclusions before I read any of the literature on the subject.

I have confined my study of cell multiplication in *Dilepis* to the sub-cuticula. In this tissue I have searched in vain for a single clear case of mitosis or amitosis. Moreover, in order to be certain I had not overlooked any, I counted 10,000 resting nuclei in the sub-cuticula of the neck regions of ten worms with the same result. Certainly active growth must have been taking place in this region, but it could not be accounted for by mitotic or amitotic division.

I have, however, observed numerous places in this region in which active cell multiplication was apparently taking place. Here multinucleate cells, such as shown in Fig. 1, have been observed. In addition to these, large protoplasmic masses were present, which varied in size from that of a single cell to that of perhaps fifty cells massed together. Fig. 2 shows a typical mass. These masses stain rather deeply with nuclear stains, and contain from one to five nuclei.

These masses are found abundantly in the neck region of every worm I examined, and occur, although less frequently, in the body region.

By reference to any of these figures it is seen at once that the mass of cytoplasm is out of proportion to the mass of the nuclei. Moreover, I have observed numerous lobes and occasionally even entire masses in which I was unable to find any trace of a distinct nucleus. Fig. 7 shows a lobe,<sup>1</sup> *i*, and Fig. 6 a mass of

<sup>1</sup> At focal levels other than that shown in the figure the lobe was seen to be continuous with nucleate masses.

protoplasm, *h*, in which no well-defined nucleus is present. However, in this latter case the mass is so close to a nucleate mass that I cannot say positively that it is not continuous with it.

By closely examining the nuclei present in these masses, I find that the nuclear membranes are very indistinct in many cases. Fig. 2 shows a mass in which the nuclei have indistinct membranes. Also one of the nuclei, *c*, has a somewhat less distinct membrane than the other, *b*. And this latter membrane is in turn less distinct than the membranes of the nuclei in the cell syncytium above it.

Moreover, a large number of nuclei have been seen which lack membranes completely. The nucleus consisted of a "nucleolus" or "karyosome" surrounded by a clear zone. Figs. 3, 4, and 5 show "karyosomes" which lack membranes. As Child and Young have already suggested, I believe this "nucleolus" represents the chromatin material of the nucleus.

By observing the protoplasm under high magnification (2,000 diameters) it is seen that the protoplasmic strands contain many dark staining granules of various sizes and shapes. Some of these granules were as large as the "nucleoli" of the complete nuclei; others, however, were so small as to be scarcely discernible. Fig. 4 shows a mass which contains a number of varying-sized granules. Fig. 5 shows a mass which contains a number of varying-sized granules one of which, *g*, is becoming surrounded by a clear zone.

The protoplasmic masses apparently arise by the outgrowth of protoplasm from certain cells of the syncytium. Figs. 2, 3, 4, and 6, show masses of protoplasm continuous with the syncytial cells around them. In Fig. 6, the developing mass is very small and contains no definite nucleus. In Figs. 2, 3, and 4, the masses are very large and contain from one to five complete nuclei. A large number of masses have been observed varying in size between these extremes. The nuclear membranes of the nuclei in the cells from which these masses are developing, contain very small, irregular granules which stain darkly like the granules in the cytoplasm. I have insufficient evidence for or against Young's view of the "de novo" origin of these granules. The chromatin granules may arise "de novo" in the cytoplasm and

develop to complete nuclei in situ. Young bases his theory of the independent origin of granules from a cytogenic protoplasmic mass upon the following facts:

1. The occurrence of masses of granular protoplasm lacking any evident nuclei.
2. The occurrence of isolated "nucleoli" of varying size from  $\frac{1}{4}$  to 1 micron in diameter, which are usually found in the above mentioned masses of protoplasm but occasionally lie free in the parenchyma strands.

I believe, however, that these facts may be equally well accounted for by assuming the extrusion of chromidia from a mother nucleus. Masses of granular protoplasm without any evident nuclei, which occur but rarely may be explained as having been severed from parent masses after impregnation with chromidia. The occurrence of isolated "nucleoli" can be accounted for just as well by assuming the migration of chromidia from the nuclei along the strands of the cytoplasmic network, as by the assumption of their development from the protoplasm in situ.

Young, in a later paper ('13) dealing with gametogenesis, in *Tænia pisiformis* says (p. 375): "I believe that new nuclei arise either from chromidial extrusions from old nuclei, or 'de novo' in the cytoplasm. . . . The structure of the nucleus—a loose collection of chromatin bodies without a membrane—renders the extrusion of chromidia an easy matter. After their extrusion new chromatin is added and that part of the cell containing them is constricted off, to give rise in its turn to other cells. . . . It is obviously impossible to say, however, whether any chromatin granule in the cytoplasm is a chromidial extrusion or a 'de novo' formation."

Since I have seen these very small granules, all of about the same size, present in the nuclear membrane as though impeded by it in their exit, along the strands of the protoplasmic network, from the nucleus to the cytoplasm, I believe that these granules are extruded from the mother nucleus. Moreover, since I have observed granules of various shapes and sizes, many of the larger ones appearing to be composed of three or four smaller ones partly united, and since I have often seen a number of

granules clustered together, I believe that the larger granules are the result of the union of many smaller ones. Thus, I believe that the small particles of chromatin or "chromidia" are extruded from the mother nucleus. Then these "chromidia" unite here and there throughout the protoplasm to form larger granules or "karyosomes" which become surrounded by a clear zone. Finally the nuclear membrane is formed, producing a daughter nucleus. When a number of nuclei have been formed multinucleate cells are the result. Since the tissue is always a cell syncytium, constrictions of the cytoplasm around a nucleus finish the production of a daughter cell. Thus one mother cell may produce a large number of daughter cells.

#### COMPARISON WITH *TÆNIA PISIFORMIS*.

In order to compare the process of cell multiplication in *Dilepis* with that in other cestodes, Dr. Young has permitted me to examine his slides of *Tænia pisiformis*, and *Cysticercus pisiformis*. Here I have identified the protoplasmic masses in both the adult and the larva. These also contain nuclei in the various stages of formation from chromidia to complete nuclei. The young larvæ show large numbers of protoplasmic masses developing in the cell syncytium. In the older larvæ the masses often show four or five nuclei developing membranes at the same time.

#### DISCUSSION.

Cell multiplication by means of protoplasmic masses and the development of nuclei from chromidia, has, so far as I am aware, never been observed heretofore in Metazoa by anyone except Young. He has described the process as it occurs in *Cysticercus pisiformis* (Young, '08) and has noted it in some other cestodes (Young, '10) although his interpretation varies slightly from my own. I have, in the present paper given an account of it as it occurs in the sub-cuticula of *Dilepis scolecina*. It is true that chromidia have been observed in certain Metazoa, but no account of their functioning in the reproduction of the cell has ever been given previous to Young's paper on the "Histogenesis of *Cysticercus pisiformis*."

If cells are actually developing from protoplasmic masses in



the manner described, we have here an exceptional method of cell multiplication, unlike anything previously described in Metazoa.<sup>1</sup> Moreover, if future research supports this view, the present theories of the role of the nucleus in heredity will have to be greatly modified at least with respect to cestodes.

As Young has previously suggested, the explanation of such a method of cell multiplication as this may rest on the fact that the cestode is highly degenerate in most characteristics due to its long period of parasitism. In the development of cells from protoplasmic masses the nucleus passes through a cycle in which occur stages resembling nuclei of lower forms. The protoplasmic mass with its diffused nuclei in the form of chromidia is comparable to a cell of the Bacteria or of the Myxophyceæ. In certain Protozoa also, as noted by many observers, the nuclear material at certain periods diffuses throughout the cytoplasm in the form of chromidia which may give origin to secondary nuclei, and these in turn to gametes. It is possible that the cestode nucleus has lost the power of mitotic division, accompanying the somatic degeneration of the worm due to parasitism. Richards, Harman, and others have shown, however, that we still find cell division taking place by mitosis in the sex cells and developing embryos.

#### CONCLUSIONS.

I have made the following conclusions in regard to cell multiplication in the sub-cuticula of *Dilepis scolecina*.

1. After a careful examination, and after counting 10,000 of the nuclei in this region, I conclude that the growth of the sub-cuticula cannot be accounted for by mitotic or amitotic division.

2. Tissue growth is taking place rapidly in this region by the development of protoplasmic masses. My reasons for believing this are the following:

- A. The nuclei in the multinucleate cells are frequently seen crowded together as if they had developed in protoplasmic masses.

- B. In the protoplasmic masses the quantity of cytoplasm is out of proportion to the number of complete nuclei present.

- C. Developing nuclei have been actually observed in the cytoplasm. The different stages of nuclear formation are shown by the following:

<sup>1</sup> A similar process was suggested long ago by Schleiden and Schwann.

- (a) The chromidia, or diffused nucleus.
- (b) The irregular chromatin granules formed by the union of numerous chromidia and surrounded by a clear zone.
- (c) The nuclear membranes of the nuclei in the masses vary considerably from delicate, scarcely discernible membranes to heavy, well developed ones.

D. These masses appear to arise by the simultaneous growth of cytoplasm and chromidial extrusions from the nuclei of certain cells.

3. The degenerate character of the nucleus is perhaps the result of the parasitic habit of the cestode.

I wish here to express my sincere thanks to Dr. R. T. Young, whose valuable criticisms and suggestions made this work possible. I also wish to express my indebtedness to Dr. B. H. Ransom for identifying my material.

#### LITERATURE.

Child, C. M.

- '07a Studies on the Relation Between Amitosis and Mitosis. BIOL. BULL., Vol. XII., pp. 89-114.
- '07b Ibid., Vol. XII., pp. 175-224.
- '07c Ibid., Vol. XIII., pp. 138-160.
- '07d Ibid., Vol. XIII., pp. 165-184.
- '10 Ibid., Vol. XVIII., pp. 109-119.
- '01 Ibid., Vol. XXI., pp. 280-296.

Harman, Mary T.

- '13 Method of Cell-Division in the Sex Cells of *Tænia teniæformis*. Journ. Morphol., Vol. XXIV., pp. 205-242.

Richards, A.

- '11 The Method of Cell Division in the Development of the Female Sex Organs, of *Moniezia*. BIOL. BULL., Vol. XX., pp. 123-179.

Young, R. T.

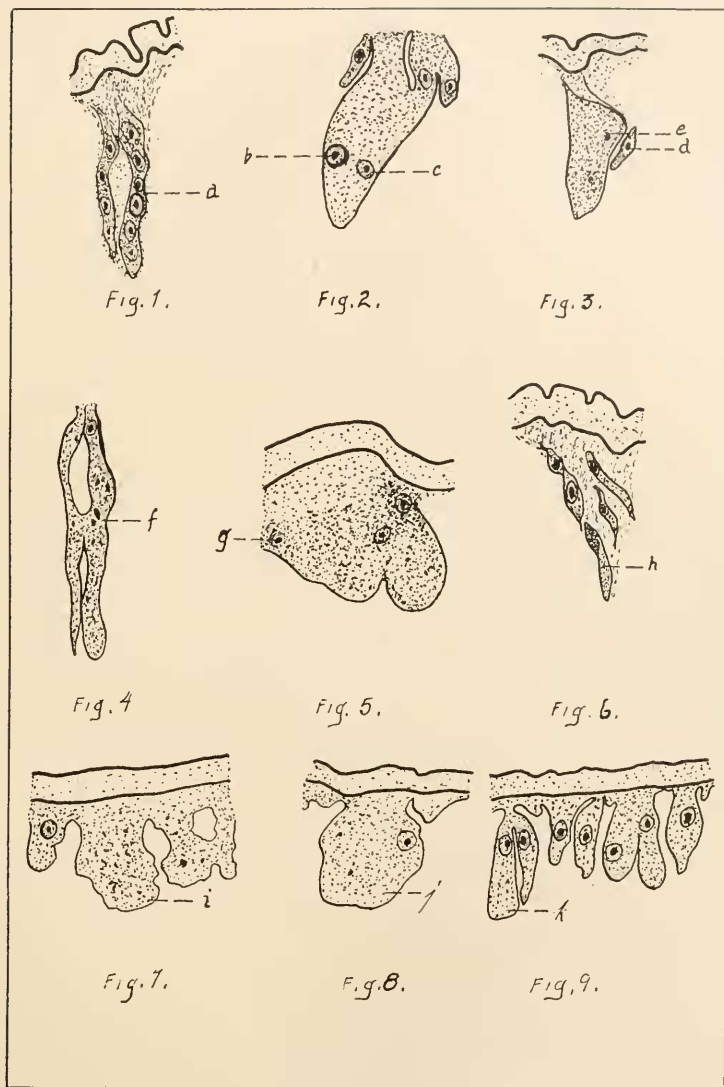
- '08 The Histogenesis of *Cysticercus pisiformis*. Zool. Jahrb. (Anat. und Ont.), Vol. XXVI., pp. 183-254.
- '10 The Somatic Nuclei of Certain Cestodes. Archiv. für Zellforschung, pp. 140-164.
- '13 The Histogenesis of the Reproductive Organs of *Tænia pisiformis*. Zool. Jahrb. (Anat. und Ont.), Vol. XXXV., pp. 355-419.





## EXPLANATION OF PLATE.


- FIG. 1. Multinucleate cell, *a*.  
FIG. 2. Nuclei with indistinct membranes, *b* and *c*.  
FIG. 3. Nuclei, *d* and *e*, lacking nuclear membranes.  
FIG. 4. Chromatin granules, *f*, in the cytoplasm.  
FIG. 5. Large chromatin granule, *g*, in cytoplasm.  
FIG. 6. A developing protoplasmic mass, *h*, in which no definite nucleus is present.  
FIG. 7. A lobe, *i*, of a protoplasmic mass in which no definite nucleus is present.  
FIG. 8. A large protoplasmic mass in the body region which contains only one nucleus, *j*.  
FIG. 9. Protoplasmic masses, *k*, developing in the body region.









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